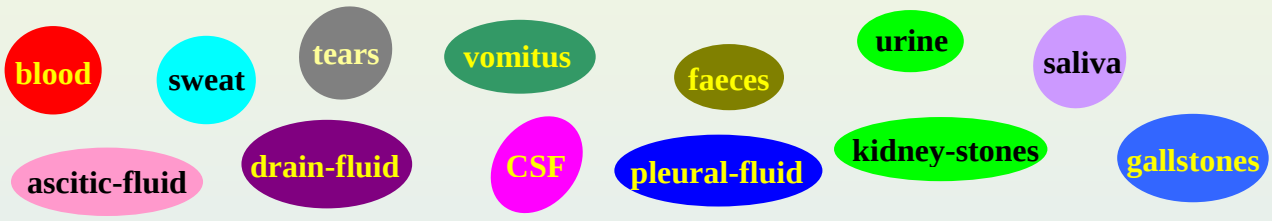
Chemical Pathology Handbook

Chemical Pathology, East Surrey Hospital, Canada Avenue, Redhill, RH1 5RH.

For use in connection with this lab. only.

Edition 4 14th Jan. 2014

INDEX See organisation of handbook & links.



PROLOGUE See organisation

This is actually the 7th edition of the Handbook if the first three booklet-forms are included. They are not, because they were too slow for the clinical setting. The latest edition has > 5k hyperlinks (many are short-cuts to related topics) for fast navigation. Researching, writing & up-dating the beast is time consuming & only adds to a workload 170 % above Royal College of Pathologists guidelines, not the easy 35 % enjoyed by others.

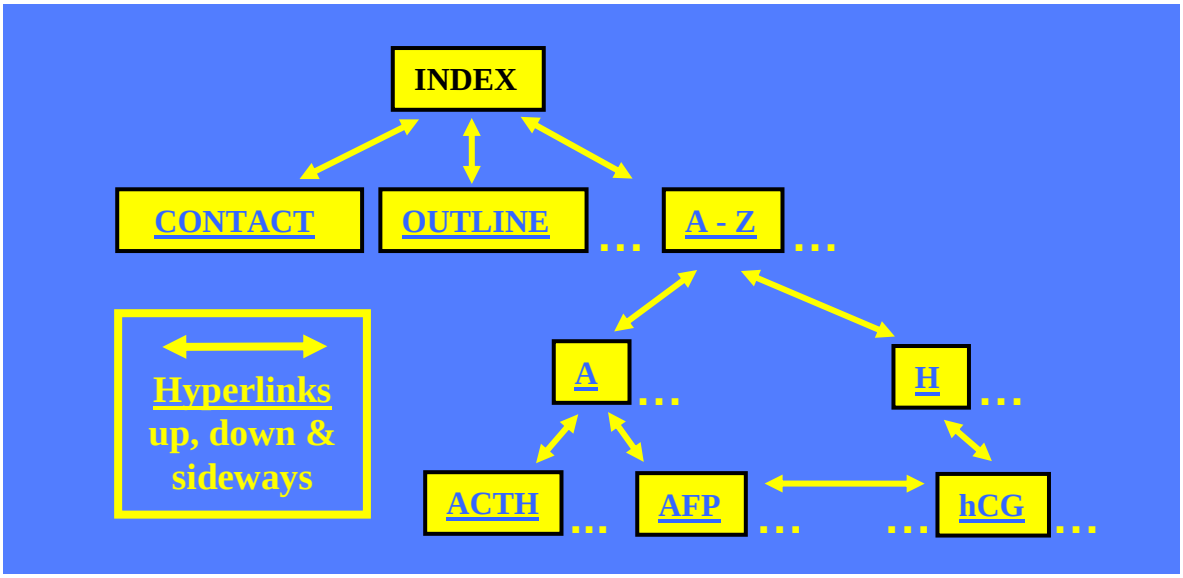
Sadly this is the last edition because new projects beckon, 90 % of the work is done at home & I cannot afford another divorce. It remains a pdf.doc because this format is fast, robust, independent of internet-vagaries & with a pdf-reader app., it can be used on mobile devices. For users who need a paper version, it is just a click away with pdf. However, the days of stand-alone handbooks are numbered & future developments will see on-screen results & requests link directly to guidance without need to search compiled versions. Can you believe I was told that this would make Pathology look complacent? Certainly not Chemical Pathology.

I would like to thank colleagues, especially in Primary Care, who have contributed ideas & expressed appreciation of the Handbook. It was written initially for community-users, but has grown into something for everyone in both primary & secondary care eg. doctors, nurses, midwives, secretaries & students. Farewell.

Dr. Robert S. Jackson

ORGANISATION OF HANDBOOK & HYPERLINKS

See Prologue & handbook story.



HANDBOOK STORY

See organisation

Aims:

- ↑ **communication** with Primary Care
- ↑ **communication** within SASH NHS Trust
- ↑ **awareness** of lab. services
- ↓ **time** on the phone
- ↓ **waste** eg. inappropriate requests
- ↓ **errors** eg. taking the wrong sample
- ↓ **time** spent writing comments on reports
- **enable users** to choose & interpret tests themselves
- **fast** enough to use in the presence of patients
- **educational**
- **entertaining**
- **suit all users** – Drs., nurses, secretaries, med. students…

Background

- **241 pages in electronic format (pdf)** which have evolved from 7 sheets of paper.
- **Fast, intuitive & heuristic navigation** via large-scale hyperlinking & redundancy.
- **No need for training** & wasting time yoyo-ing back & forth to a root menu.
- **Save as a pdf.doc on your HD** to escape internet-vagaries. Get a pdf reader app. for your mobile device.
- **A printed version** is only a click away.

Feedback

I avoided a referral by double-checking with your handbook.

.. I shall certainly use it & recommend it to all those I teach.

..well presented…

..very interesting..

..I thoroughly enjoyed looking at it..

..clear, concise, informative..

..entertaining..

..very helpful indeed...

..very detailed & useful information..

...all levels at our practice benefit..

..very user-friendly..

..much appreciated..

CONTACT

Contact	Name	tel. & bleep	email
East Surrey Hospital, Canada Ave., Redhill, Surrey, RH1 5RH		01737 768511	FAX. 01737 231694
OUT-OF-HRS BIOMEDICAL SCIENTIST (BMS)		**bleep 553**	
Pathology Reception 8 – 8 Mon.-Fri. 9 – 8 Sat. & Sun.		ext 1691	
Reception Manager	Ms. Carol Moon	ext 1691	Carol.Moon@sash.nhs.uk
Pathology Services Manager	Mr. Mike Rayment	ext 1894	Michael.Rayment@sash.nhs.uk
Quality Manager	Mr. Mike Rayment	ext 1894	Michael.Rayment@sash.nhs.uk
Computing Manager	Mr. Martin Stone	ext 6467	Martin.Stone@sash.nhs.uk
Phlebotomy Manager	Ms. Kerri Campbell-Bowler	ext 6444	Kerri.Campbell-Bowler@sash.nhs.uk
Lead BMS Chemical Pathology	Mr. Arnold Rady	ext 1819	Arnold.Rady@sash.nhs.uk
Lead BMS Haematology	Mr. Eddie Onipede	ext 2192	Edward.Onipede@sash.nhs.uk
POCT Officer	Ms. Denise Anderson	ext 6456	Denise.Anderson@sash.nhs.uk
Cons. Chemical Pathologist	Dr. Robert S. Jackson	ext 1693	Robert.Jackson@sash.nhs.uk
Pathology Stores	Mr. Gabriel Alvarez	ext 6447	PathStores@sash.nhs.uk

OUTLINE OF CHEMICAL PATHOLOGY section of Integrated Blood Sciences. See A-Z

High workload 4.1m tests in 2012 Up 6.1 %. Demand-control kept it below the 6.2 % ↑ of ’11.

High productivity

Shift working enables a **wide repertoire of tests** 24/7 to expedite completion of patient episodes.

Below average costs

Fast A/E results, 94% within 60 mins.

Full accreditation

CPA

Lab. ref. 0537

Service to the private sector, routine & back-up

An **Integrated Blood Sciences Lab.** with Haematology & Blood Transfusion to ↑ efficiency.

Fully accredited in both Chemistry & Haematology

Electronic requesting & reporting of results to GPs & hospitals.

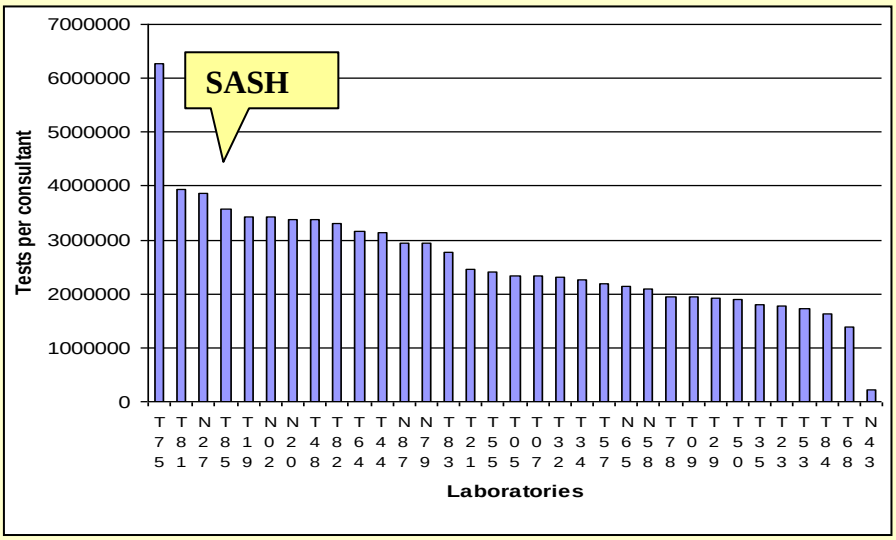
Uninterrupted service using duplicated Roche Cobas 8000, main & immunoassay analysers.

Single-site efficiency. Crawley & ESH labs. were integrated at ESH in Nov. 2005.

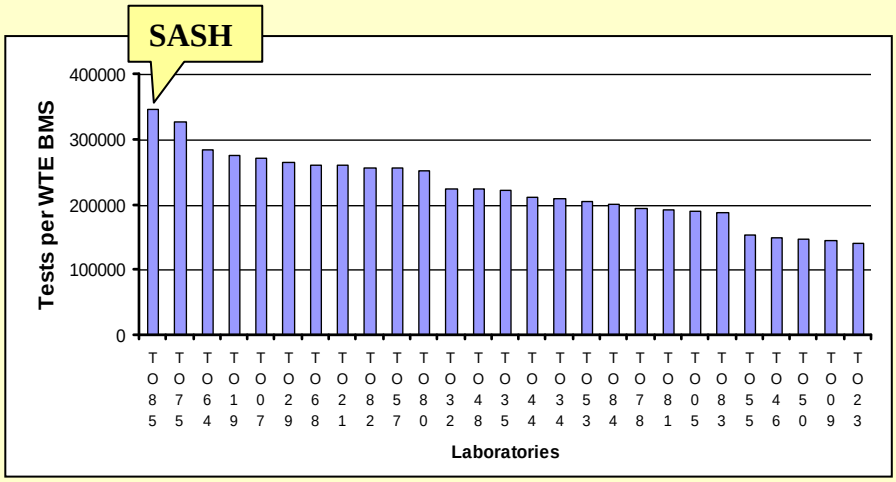
Excellent training of Surrey University BSc BioMedical Science students. “The Training Officer Andrea was very efficient”. “The ESH programme is clearly very successful & should be shared across the network” – Past trainees.

Teaching rated as excellent by BSMS med. students.

PRODUCTIVITY See costs, shift working & workload.

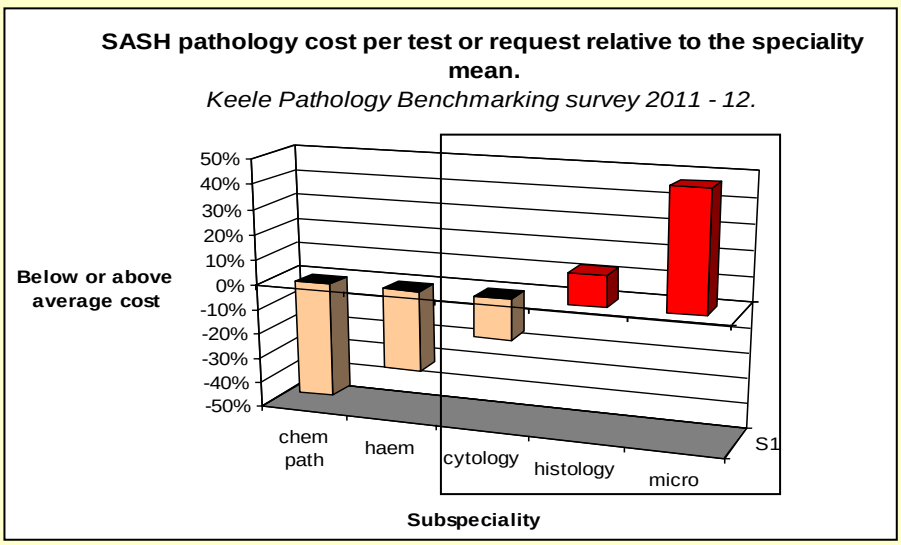


SASH Chemical Pathology has the 4th most tests per consultant per year
Keele Review 2012



and *the* most tests per BMS per year.
Keele Review 2012

COSTS See shift working, photographs, productivity & workload.

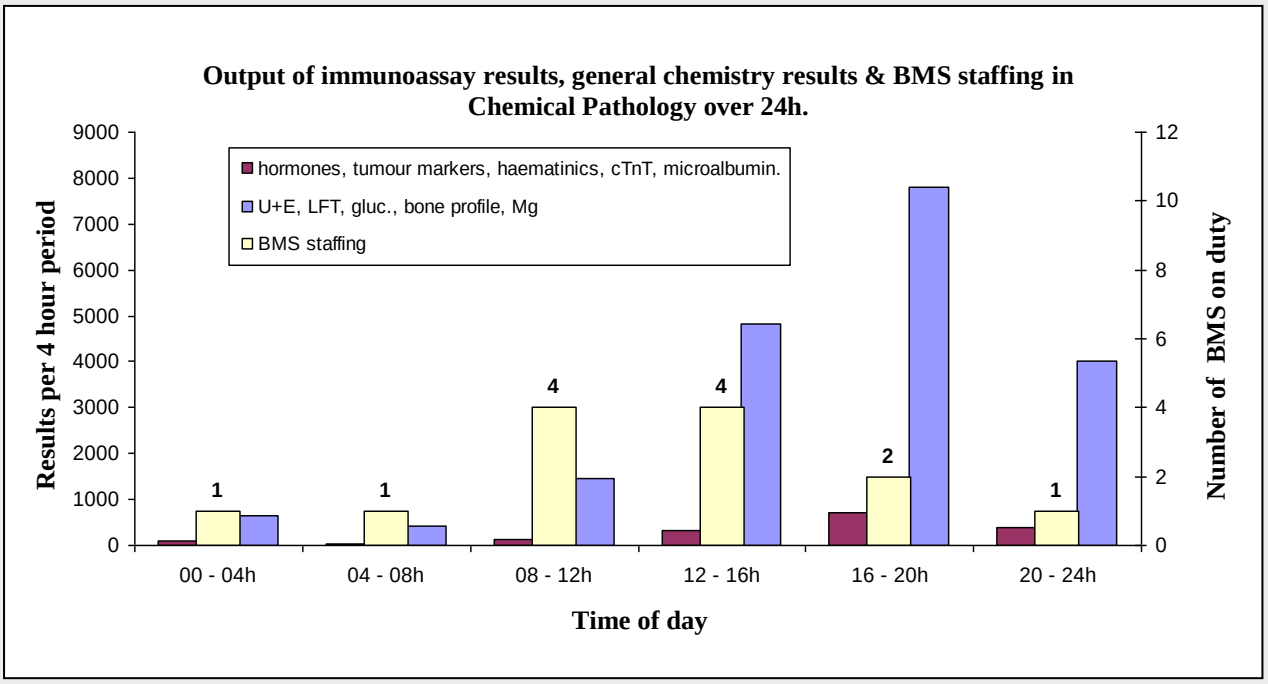


The costs of tests in the IBS sub-specialities are well below average.
Keele Review 2012

SHIFT WORKING See costs, productivity & workload.

Shift working **enables:-**

- **Most tests to be available 24/7** to expedite patient management & discharge.
- **Results to be ready asap on late samples** from OP clinics & GPs.
- **Efficient usage of investments** by maximising analyser loading round-the-clock (Carter 2008).
- **Minimal service-disruption** for analyser-maintenance by timing it when outstanding work & demand are low:



AVAILABILITY OF SERVICE

See reception

Mon. – Fri. 8am – 8pm

Full service if samples are in the lab. by 7pm.

Sat. & Sun. 9am – 1pm

Full service if samples are in the lab. by noon.

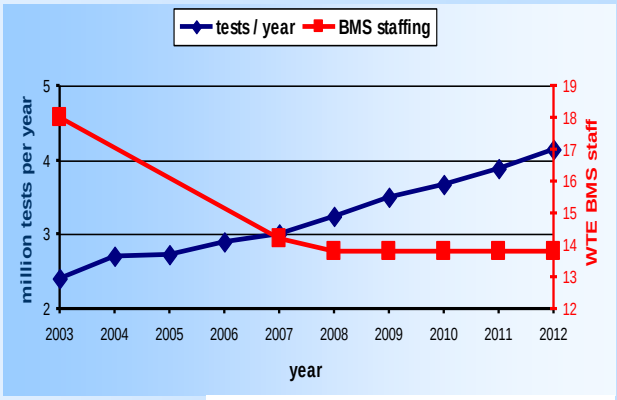
Out-of-hours

- **Just send** the samples
- Analysed as they arrive.
- **Bleeping or phoning is unnecessary & causes delay.**
- Results are on the computer in about 1h.
- Some tests are too costly & infrequent to offer 24/7 eg. prolactin, but they can be run if necessary. **Please ask**

WORKLOAD - high & rising in support of expanding NHS services.
See Co, costs, outline, productivity & shift working.

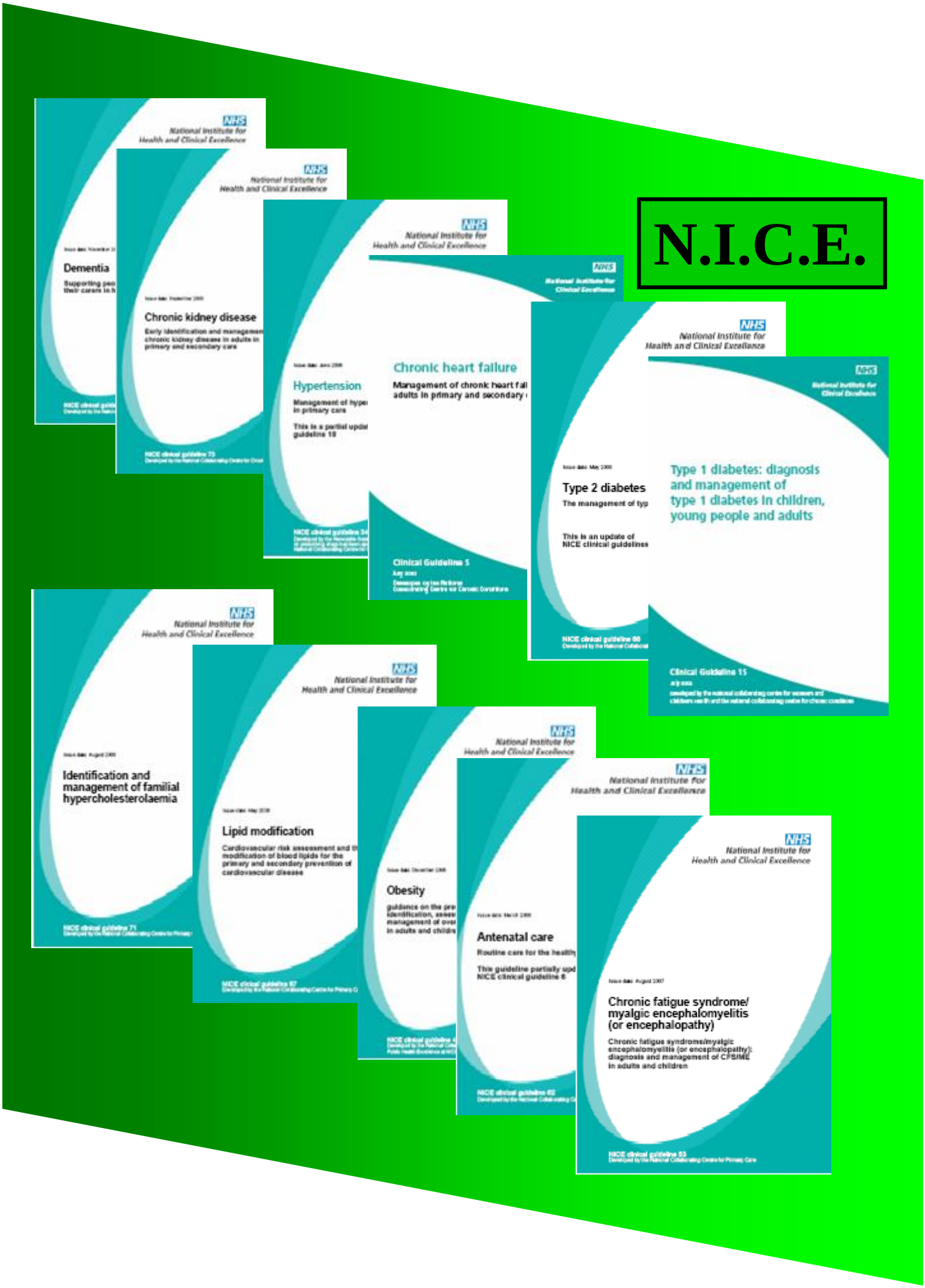
Causes of ↑:

- **Time limits** eg. 4h in A/E & 18 w to treatment, discourage discrete & stepwise requesting.
- ↑ patient expectations / **zeitgeist**.
- **Social changes** eg. fertility expected later in life.
- ↑ **obesity** with ↑ diabetes, hypertension, cholesterol, liver & ♀ & ♂ hormone problems.
- ↑ **alcohol** related diseases.
- ↑ **management of complex diseases** in general practice eg. diabetes & CKD.
- ↑ **elderly** population.
- ↑ **access to therapies** eg. renal dialysis.
- ↑ **consultants** eg. cardiology, gastroenterology & endocrinology.
- **Rewards** for requesting: **QOF** (Quality & Outcome Frameworks) points.
- **NSF** (National Service Frameworks).
- **NICE** (National Institute for Health & Clinical Excellence) guidelines.
- **New tests** eg. faecal calprotectin.
- **Learned advice** eg. testo. assay in DM by the Endocrine Soc.
- **MHRA** (Medicines & Healthcare Products Regulatory agency) advice.



ie. **in 10 yrs:**

- tests ↑ 71%
- BMS staff ↓ 24%
- 1 consultant no change (RCPath recommends 2.7)
- no biochemists

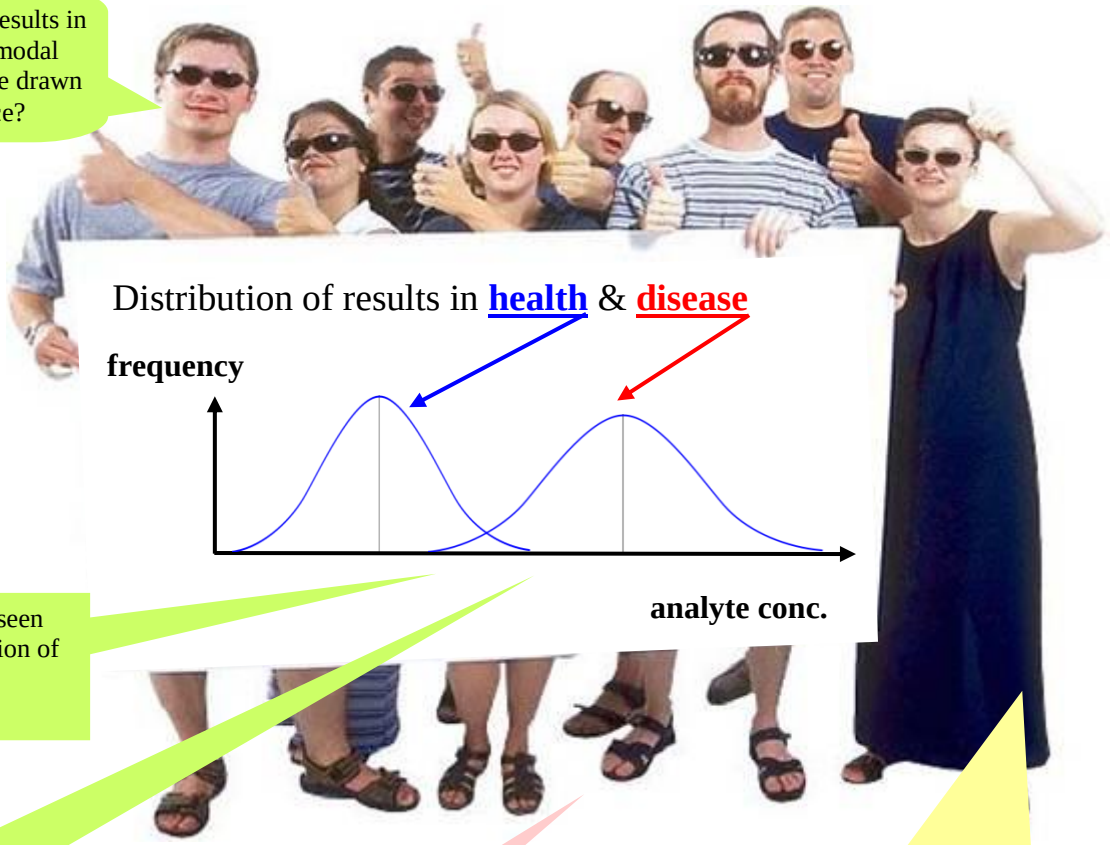

N.I.C.E.
National Institute for Health and Clinical Excellence
Dementia
Chronic kidney disease
Hypertension
Chronic heart failure
Type 2 diabetes
Type 1 diabetes: diagnosis and management of type 1 diabetes in children, young people and adults
Clinical Guideline 15
Identification and management of familial hypercholesterolaemia
Lipid modification
Obesity
Antenatal care
Chronic fatigue syndrome/ myalgic encephalomyelitis (or encephalopathy)

WHAT'S A REFERENCE RANGE? - **Not a normal range**, only a guide.

Ref. range problems have increased with the survival of people at the extremes of age & the ability to do so many tests on tiny samples. Ref. data in people >80y, children & preterm neonates are limited, especially with new assays. If many elderly people are never without medical problems or therapy, how appropriate are ranges generated in young disease-free people eg. blood-donors?

Ref. ranges are a compromise of factors eg. sensitivity, specificity, the consequences of false conclusions, disease prevalence, how much the distributions overlap, whether they are parametric or nonparametric, skewed, kurtotic…..

Why not a normal range? Results in health & disease are rarely bimodal (separate). So where is the line drawn to make a practicable reference?



If a ref. range excludes values seen in illness, sensitivity for detection of disease ↑ (↓ false negs), but specificity ↓ (↑ false pos).

If a range includes all results seen in health, specificity ↑ (↓ false pos), but sensitivity ↓ (↑ false negs).

Ref. ranges only suggest increasing likelihood of abnormality as the limits are approached or passed. They do not define normality & results must always be seen in their clinical context.

….thus there are multiple ways of defining references:

- 2.5th – 97.5th centile values (mean ± 2SD if distribution's Gaussian) ie. 1/40 normals have results ≤ the lower limit & 1/40 > upper limit, eg. *FT4*.
- 5th – 95th centile values ie. 1/20 normals have results ≤ the lower limit & 1/20 > upper limit, eg. *progesterone*.
- thresholds, if the lower limit in normals is undetectable or insignificant eg. *CA19.9* (97.5th c.), *cardiac troponin* (99th c.)
- advisary data eg *cholesterol* & *therapeutic drug monitoring* if disease risk extends throughout a simple descriptive ref. range or clinical evidence of health or illness is unclear.

GETTING RESULTS

- Ultimately, it is the **requester's responsibility** to look for results.
- If a significant result is not sought, counsel might ask "Surely doctor, you would not have requested a test without good reason? Why then did you not look for the result?"
- This obligation creates risk in "**defensive requesting"** where large numbers of results are generated, each one of which might be significant & must not be overlooked.

Electronic

- Most results are now sent electronically to GPs & hospital staff.

Paper

- Paper reports are **not provided routinely** because delivery may fail eg. due to unclear writing on the request form causing loss of location or consultant details.

Telephone

See telephoned results.

- Please avoid calling - risk of errors & it slows the lab.
- Please **check the computer before phoning**.

TELEPHONED RESULTS

- Results are telephoned if they need **urgent attention**.
- Effective contact details MUST be given on the request form

Action-limits for telephoning

Adapted from RCPath 2010.

	phoned to ward or surgery		phoned directly to doctor		
	under	over	under	over	
sodium	125	150	120	155	mmol/L
potassium	3.0	6.0	2.0	6.5	mmol/L
urea		25.0		40.0 10.0	mmol/L if <16y old
creatinine		350		600 200	umol/L if <16y old
glucose new cases known DM		16.0 25.0	2.5 (adults) 2.0 (children)	30.0 30.0	mmol/L mmol/L
amylase		300		1000	U/L
calcium	1.80	3.00	1.60	3.50	mmol/L
magnesium	0.5	1.5			mmol/L
lithium		1.2		1.5	mmol/L
cortisol			100		nmol/L
digoxin		2.5		3.1	ug/L
phenytoin		23		30	mg/L
carbamazepine		15		20	mg/L
gentamicin		10.0			mg/L
vancomycin		15.0			mg/L

TURN-AROUND TIME

The time taken to produce a result depends on **many factors** eg.

- Time to **reach lab**. (outside our control).
- 15 min. in **centrifuge**.
- 18 min. **incubation** for immunoassays eg. cTnT.
- **More tests** per request = longer to complete.
- **Queuing** when the analyser is busy eg. in afternoon.
- Analytical **quality checks**.
- **Double-checking** of abnormal results.
- Re-assay of samples in **dilution** when the initial result is extreme.
- Performance of **additional tests** as indicated by the initial results.
- **Maintenance** – daily, weekly & monthly procedures.
- **Breakdowns**.
- Low-demand tests are **batched** & not performed every day.
- Results for tests **sent** to other labs. take 1 – 4 weeks to arrive.

Targets for turn-around times.

TURN-AROUND-TIME			
A/E	**In-patients**	**Community**	**TESTS**
< 1 h	**< 2 h**	**< 1 d**	amylase • bicarb • blood gas • bone profile • CK • CRP • CSF protein + glucose • GGT • glucose • LFT • Mg • paracetamol • salicylate • cTnT • U+E
< 2 h	**< 3 h**	**< 1 d**	AST • carbamazepine • digoxin • direct bili • Fe studies • gent. • hCG • lactate • LDH • lipids • Li • phenytoin • theophylline • urate • valproate • vanc.
> 3 h	**> 3 h**	**< 1 d**	B12 • cortisol • ferritin • folate • other hormones • TFTs • "tumour markers".
1 – 4 w	**1 – 4 w**	**1 – 4 w**	Tests sent to other sites

REQUESTING • give relevant clinical details • ↓ risk to the patient & yourself

- Request electronically if possible.
- Use **demographic stickers on forms**
- Write patient details on sample tubes **before leaving** the patient.
- **Avoid ambiguous terms** eg. "baby of….", "husband/partner of….".
- Complete **all fields**.
- Give **relevant drug & clinical info.** eg. on T4, amiodarone, PPIs, pregnant, hypopit., which enable the lab. to add tests & minimise inappropriate ones.

- **USELESS DETAILS** eg. blank, fullstop, unwell, screening, monitoring, routine, repeat, revue, pre-op, post-op, follow-up, down /up last time, Dr./consultant/patient wants it…..

- Give **contact details** so that the lab. can contact you re. request or result problems.
- **Reference centre tests** eg. renin & Cu, **MUST** have relevant clinical details eg. drugs, histo. diagnosis

SAMPLE TUBES, VOLUMES & PROFILES See A – Z

tube	details
	No anticoagulant. Separation gel & clot activator present.4mL. **1st tube**
	No anticoagulant, separation gel, or clot activator. 5mL **2nd tube**
	K EDTA. 4mL **3rd tube**
	Potassium fluoride + sodium oxalate. 2mL **4th tube**
	Lithium heparin. 6mL **5th tube**
	Na heparin. No separation gel. 6mL. Zn, Al & trace metals. **6th tube**
	Plain 24h urine bottle from Path Reception. Any acid needed is added by lab. on its return.
	Sterile plain universal container

cap ring	meaning
	yellow ring = with separation gel
	black or no ring = no separation gel
	white ring = less blood drawn. Paediatric use.

Sample tubes See A – Z

- Collect tubes in the **correct order** to avoid contamination errors eg. ↑ K from K EDTA
- **Fill** "to the line"
- **NEVER decant** blood from 1 tube to another. Dangerous errors can be produced.
- **Be gentle**. Would you hoover a hamster? Blood contains delicate cells too.
- Always write at least the **full name & d.o.b.** on the sample **before leaving** the patient.

Volumes See A – Z

- The **minimum volume** of **whole blood** is indicated in the A-Z section, thus eg. 1.5 mL
- Includes allowance for haematocrit, assay vol., duplication, dilution, wetting & deadspace.
- Smaller vols. are possible, but there may not be enough to double check the result eg. in dilution
- **Some tests need their own tube eg. PTH**
- See below for vol. needed for **profiles**

Profiles See A – Z

- Common tests can be requested in groups eg. U+E.
- By sharing the "wastage" vol., the sample vol. for a profile is less than the sum of the separate tests.
- These profiles need **1mL of blood** ✚ the vol./profile eg. U+E + LFT + SPE = 1 + 0.5 + 0.5 + 2.0 = 4.0 mL

	mL
• U+E+creatinine, bicarbonate, chloride	0.5
• LFT (total & direct bil., ALT, ALP, alb., GGT)	0.5
• bone (Ca, PO4, Alb., ALP)	0.5
• TFT	0.5
• haematinics (B12, folate, ferritin, iron studies)	1.0
• "tumour markers" (CA125, 153, 199, CEA, AFP)	1.0
• therapeutic drugs	1.0
• lipid profile (Tg, total, HDL & LDL chol.)	1.0
• FSH, LH, prolactin, E2, testo., cortisol, SHBG	1.0
• serum protein electrophoresis (SPE)	2.0

ADDING TESTS to a previous request.

- Samples are **kept for 3 – 4d**, space permitting.
- **Tests will not be added to specimens over 3 d old.**
- Some analytes degrade sooner eg. vitamin B12 & folate
- The latest that tests can be added is indicated thus 3 d in the A-Z listing.
- **Request form needed.**
- Procedure:

SASH
- get a form: ➔
  Intranet > Departments > Cerner > Pathology Order Comms > Pathology Add on Sample Request > print
- fill it in
- send to lab.

GP
- order tests on ICE as usual
- enter **ADD ON TEST** as the clinical detail
- print form
- send to lab.

PATHOLOGY ADD ON REQUEST
DATE:
ORIGINAL SAMPLE DATE:
SAMPLE No. (Right click on order & select order information, click onto additional information tab & insert the Accession Number here)**:**
PATIENT DETAILS: (Stick Patient label here) **SURNAME** **FORENAME** **DOB**
TEST REQUIRED: NOT TO BE USED FOR MICROBIOLOGY TEST REQUESTS.
CONTACT EXT NO/BLEEP:

DYNAMIC ENDOCRINE TESTS.

LHRH, TRH & Synacthen tests can be combined.

LHRH test
TRH test
short Synacthen test (SST)
overnight dexamethasone suppression test (ONDEX)
2 day dexamethasone suppression test
5 day dexamethasone suppression test
growth hormone suppression test (OGTT)
glucagon stimulation test

Luteinising Hormone Releasing Hormone (LHRH) test.

See FSH, LH, oestradiol, testosterone & TRH test.

- **Fasting unnecessary**.
- **Follicular phase** of menstrual cycle (d 3 – 7) ideally

Use & Test

- Ix **hypothalamic/pituitary deficiency.**

- Low sensitivity & specificity ie. **basal FSH & LH** are usually enough (see below).

- Ix **precocious puberty**, **delayed puberty** & **premature thelarche**.
- LHRH 100 ug iv. (adult). Response affected by age, gender & maturity, menstrual phase, pathology & Px.
- Can combine with other dynamic tests eg. TRH test.

Assays & sample-times

0 min (just before LHRH iv)	**30 min** after	**60 min** after
LH	LH	LH
FSH	FSH	FSH
testosterone or oestradiol		

Background

LHRH is a decapeptide secreted in the median eminence by axons from neurons in the hypothalamic pre-optic area. The hypophysial portal blood flow carries it to the gonadotropes of the anterior pituitary which secrete LH & FSH in response. LHRH is trophic ie. chronic deficiency in hypothalamic disease results in gonadotrope atrophy. Administered synthetic LHRH (Gonadorelin) can stimulate LH & FSH secretion by a normal non-atrophic pituitary.

References Guidance only. **Peak usually at 20 – 30 min**

		0 min. Just before LHRH	**30 min.** after LHRH	**60 min.** after LHRH
men	**LH**	1.7 – 8.6 U/L	11 – 48 U/L	13 – 58 U/L
	FSH	1.5 – 12.4	1 – 5	1 – 7
women	**LH**	2.4 – 12.6 (fol. phase)	12 – 35	15 – 42
	FSH	3.5 – 12.5	1 – 25	1 – 11

Interpretation Always relate results to the **clinical findings**.

- **Pre-pubertal females**: LH peak < 5 & FSH peak > LH peak.
- **Pubertal females**: LH peak > 5 & increases with maturity. LH peak > FSH peak.
- **Post-pubertal females**: LH ↑ 4 – 10x as above. LH response exceeds FSH ↑.
- **Males**: similarly, but LH response exceeds FSH ↑ at all ages.
- **Menstrual cycle**: Luteal phase LH ↑ is nearly 3x the follicular one. Much less difference with FSH.
- **Precocious puberty**: gonadotropin independent. Basal LH & FSH ↓. LHRH response ↓.
- .. .. .. dependent. Basal LH & FSH usually ↑. LHRH response ↑.
- **Primary gonadal failure** eg. menopause & Kleinfelter's syn., causes an **exaggerated** response. Basal FSH & LH are usually sufficiently ↑ to be diagnostic & the LHRH test is unnecessary.
- **2ndy gonadal failure** (hypogonadotropic hypogonadism):
  HYPOTHALAMIC disease: **normal or ↑** response**.** Priming with LHRH may be needed to restore responsiveness to atrophic gonadotropes.
  PITUITARY dis.: ↓ response. In reality, the LHRH test is weak at distinguishing these disease.

Thyrotropin (TSH) Releasing Hormone (TRH) test.

See FT3, FT4, LHRH test, prolactin & TSH.

- **fasting unnecessary.**
- **any time** of day or menstrual cycle.
- **can combine** with other tests eg. LHRH, but note their preconditions.

Use

- **Ix of hypothalamo/pituitary deficiency**. High sensitivity TSH assays can distinguish levels suppressed by chronic thyroid hormone excess (< 0.1 mU/L) from low-normal ones & have replaced the TRH test for diagnosis of primary hyperthyroidism.
- **Ix ambiguous TFTs.** Rare use because TFTs of 2ndy hypothyroidism are usually accompanied by obvious deficiencies in other pituitary functions.

Test iv. TRH (200 ug adult, 1 ug/kg <12y). See the BNF.

Background TRH is a tripeptide (3 aa) secreted in the median eminence by axons from neurons in the hypothalamic paraventricular nucleus. It is carried by the hypothalamo/pituitary portal blood flow to the thyrotropes of the anterior pituitary where it stimulates secretion of thyroid stimulating hormone (TSH).

Assays & sample times

0 min just before TRH iv	**20 min** after TRH	**60 min** after TRH
TSH	TSH	TSH
FT4	Do not measure FT4 & FT3. Levels cannot change from basal on this short time scale.	
FT3 (if needed)		
prolactin (if needed)	(if needed)	(if needed)

References - A TSH peak of 5 – 22 mU/L at 20 min.

Interpretation

- **Primary hyperthyroidism** – ↓ basal TSH & ↓ response.
- **Primary hypothyroidism** – normal or ↑ basal TSH with ↑ response
- **Pituitary** disease (2ndy hypothyroidism) – ↓ or "normal" basal TSH & ↓ or no response.
- **Hypothalamic** disease – ↓ basal TSH with delayed peak response ie. 60 min value > 20 min one.
- **Acromegaly & Cushing's** – TSH response may be ↓ despite euthyroidism.

PROLACTIN levels ↑ after TRH, but the clinical value of this pharmacological effect is minor.

- In health ↑ about 5x by 20 min.
- Prolactinomas show ↑ basal PRL & the response to TRH may be ↓.
- ↓ hypothalamic inhibitory control of PRL secretion causes ↑ basal PRL & ↑ response to TRH.
- **Drugs** ↑ response with oestrogen, theophylline & l-dopa.
  ↓ .. corticosteroids.

short Synacthen test (SST). Synacthen is the tradename of ACTH (1-24).

See ACTH, comments, cortisol & 17-OHP.

- **fasting unnecessary.**
- **9am** but not essential.

Use & Test

- Ix **primary adrenocortical deficiency** (Addison's dis.). 250ug Synacthen iv in adults (see BNF).
- Ix **hypothalamic/pituitary disease** ie. 2ndy adrenocortical deficiency. Adrenocortical atrophy due to ↓ ACTH secretion, is reflected in the Synacthen test. 250ug is an excess & false – ve results can occur. 1ug is more physiological & the results correlate better with those of the insulin stress test, the "gold-standard".
- Ix **congenital adrenal hyperplasia** (CAH). If the results of spot-tests are inconclusive, abnormalities of adrenocortical steroid intermediates in CAH can be accentuated by stimulation with Synacthen eg. assay 17 hydroxyprogesterone with cortisol in suspected 21-hydroxylase deficiency.

Background See ACTH

Assays & sample times

0 min (just before Synacthen iv)	**30 min** after Synacthen	**60 min** after Synacthen
Cortisol	cortisol	cortisol
ACTH *		

* Plasma ACTH is sometimes added on the grounds that it will be ↑ in primary adrenocortical hypofunction & ↓ in 2ndy failure. See ACTH. In reality, the contribution to diagnosis is degraded by considerable overlap of results due to analytical, physiological & disease variation. However, in **paediatrics** especially, ACTH assay may help to exclude the need for stress testing if the results are interpreted with due caution.

References

- 30 min serum cortisol > 500 nmol/L.
- Increment > 250 nmol/L (may be less if the basal cortisol is high).
- Borderline results: caution, consider risk & clinical context. Repeat if necessary.

overnight dexamethasone suppression test (ONDEX).

See ACTH, comments, cortisol (serum), cortisol (urine) & 2 day & 5 day dex. suppr. test.

Use & Test For screening for Cushing's syndrome. The ONDEX is simple & more reliable than spot tests.

- 1 mg dexamethasone p.o. at 10 - 11 pm.
- Gold cap clotted blood sample for cortisol taken next day at 8 - 9am.

Background Dexamethasone, a synthetic glucocorticoid which is not measured in the cortisol assay, should inhibit ACTH secretion by the pituitary causing ↓ adrenocortical stimulation & ↓ serum cortisol.

Reference Serum cortisol ↓ to ≤ 50 nmol/L in 95% of healthy people & ≤ 140 nmol/L in 97%.

Causes of ↓ suppression The 2 day & 5 day dex. suppr. tests may be needed to distinguish them.

- **ACTH dependent** - ectopic ACTH secretion (SCC lung, carcinoid), Cushing's dis.
- **.. independent** - adrenal adenoma/carcinoma
- **Pseudo-Cushing's dis.** - depression, alcoholism
- **↑ clearance of dexamethasone** - enzyme induction by phenytoin, phenobarbitone, rifampicin
- **Patient non-compliance** - misunderstood or forgot to take dexamethasone

2 day low dose dexamethasone suppression test.

See ACTH, cortisol (serum), cortisol (urine), 5 day dex suppr test & ONDEX.

Use & Test This, the classic Liddle test, is a more laborious screen for Cushing's syndrome than the ONDEX, but it suffers **fewer false + ves** eg. due to depression, ethanol or obesity. **Background** - see ONDEX.

- 0.5 mg dexamethasone x 8 doses po., strictly 6 hrly, starting at 9am day 1.
- Take blood for serum cortisol at 9 am on days 1, 2 & 3.

Schedule of assays & dex. doses.

	day 1				day 2				day 3
time	9am	3pm	9pm	3am	9am	3pm	9pm	3am	9am
dex.	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g	
sample	cortisol				cortisol				cortisol

Reference & interpretation

- Cortisol should fall to ≤ 50 nmol/L, even in the stressed groups above, but not other cases of Cushing's syn.
- **Failure to suppress** – will need a 5 day dex. suppr. test to shed light on the cause.
- **False + ves** may occur eg. failure to take the dex. & ↑ dex. clearance due to enzyme induction by phenytoin, phenobarbitone & rifampicin.

5 day high dose dexamethasone suppression test.

See ACTH, cortisol (serum), cortisol (urine), 2 day dex. suppr. test & ONDEX.

Use Ix of Cushing's syndrome

Test Essentially, the 2 day dex suppr test + repetition at higher dose to identify the cause of cortisol hypersecretion. An inpatient procedure best handled by an endocrinologist. Tests can be added eg. cortisol at midnight before the start & 24h urine cortisol measurements. Background - see ONDEX.

Schedule of samples & dex. doses.

	day 1				day 2			
time	9am	3pm	9pm	3am	9am	3pm	9pm	3am
dex.	0.5mg	0.5mg	0.5mg	0.5mg	0.5mg	0.5mg	0.5mg	0.5mg
cortisol	*				*			
ACTH								

	day 3				day 4				day 5
time	9am	3pm	9pm	3am	9am	3pm	9pm	3am	9am
dex.	2.0mg	2.0mg	2.0mg	2.0mg	2.0mg	2.0mg	2.0mg	2.0mg	
cortisol	*				*				*
ACTH	*				*				*

References

- The low dose first half should be like the 2 day dex. suppr. test ie. cortisol should ↓ to **≤ 50 nmol/L**.
- With "**ectopic ACTH**" secretion & **adrenal tumours**, cortisol fails to suppress even after high dose dex. ACTH levels helps distinguish these - ↑ in the former & ↓ in the latter.
- In **pituitary dependent Cushing's** dis., there is commonly little or no suppression after low dose dex., but at high dose, cortisol ↓ > 50%. ACTH levels help to confirm the diagnosis.
- **NB**. The reality of diagnosing the cause of hypercortisolism can be less than clear-cut!

Growth Hormone Suppression test (OGTT)

See GH, glucagon stimulation test, glucose, IGF1 & OGTT.

- **fasting needed.**

Use & Test Ix of acromegaly & gigantism. Essentially, an OGTT with extra assays.

Background

Hypoglycaemia causes ↑ secretion of growth hormone (GH), although its counter-regulatory role is relatively minor. In response to an oral glucose load (OGTT), plasma GH normally falls. In acromegaly this response may be blunted or GH levels may paradoxically increase.

Assays & sample-times

0	**30**	**60**	**90**	**120** mins.
glucose	glucose	glucose	glucose	glucose
GH	GH	GH	GH	GH
IGF1				

References & interpretation Serum GH normally ↓ to ≤ 0.7 ug/L (2 mU/L).

- In **acromegaly** serum GH may ↓ but inadequately, not at all or even ↑ (50% of cases).
- Plasma glucose is measured because acromegaly predisposes to **diabetes mellitus**.
- Serum **IGF1** is measured to confirm its value as a marker of GH secretion.

glucagon stimulation test

See cortisol, glucagon, glucose, GH, GH suppr. test, IGF1 & IGFBP3.

- **Fasting.**
- In the **morning.**

Use & Test

- Ix of deficient GH & ACTH secretion.
- Avoid with insulinomas or phaeochromocytomas.
- Glucagon given s/c *children* 0.1 mg/kg up to 0.5 mg
  adults 1.0 mg or 1.5 mg if > 90 kg
- Nausea & abdominal discomfort may occur 30 – 60 min. later.
- Patient **MUST EAT A MEAL** containing unrefined carbohydrate at the end of the test to prevent late-onset hypoglycaemia, especially with exercise eg. walking to the car park.

Assays needed

Do not measure glucagon.

0	30	60	90	120	150	180 mins.
glucose	glucose	glucose	glucose	glucose	glucose	glucose
GH	GH	GH	GH	GH	GH	GH
cortisol	cortisol	cortisol	cortisol	cortisol	cortisol	cortisol
IGF1						

Background

- Alternative to insulin-induced hypoglycaemic stress for Ix deficient GH & ACTH secretion.
  ACTH is measured indirectly as the cortisol response of the adrenal cortex.
- Glucagon causes ↑ plasma glucose in the 1st hour, which triggers insulin secretion, then ↓ plasma glucose & stimulation of GH & ACTH secretion.
  Glucagon can also directly stimulate GH secretion ie. without hypoglycaemia.

References.

- Serum GH ↑ to a peak > 6.7 ug/L (20 mU/L) at 120 – 180 mins. excludes GH deficiency.
- A weak GH response can be seen in debilitated people or even some healthy ones.
- Serum cortisol ↓ as glucose ↑ in the 1st hour, then it ↑ to ≥ 1.5x the basal level by 180 mins.

THERAPEUTIC DRUG MONITORING (TDM) See notes on TDM & comments.

Drug	Plasma half life	Time to steady state	Therapeutic range
amikacin			
amiodarone	240 - 2400h	60 - 90d	0.5 - 2.0 mg/L
N-desethylamiodarone			0.5 - 2.0 mg/L
caffeine (neonates)	40 – 230h		5 - 20 mg/L
carbamazepine	8 - 60h	7d	4 - 10 mg/L
clobazam	18h	4d	< 200 ug/L
clonazepam	18 - 50h	4 - 10d	25 - 85 ug/L
clozapine			0.35 – 0.50 mg/L
cyclosporin	8 – 20h	2 – 4d	depends on use ug/L
desmethylclobazam			< 2000 ug/L
digoxin	36h	7d	0.8 - 2.0 ug/L
ethanol			Drive limit 800 mg/L
ethosuximide	50h	10d	<100 mg/L
flecainide	20h	5d	0.15 - 0.90 mg/L
gabapentin	6h	2d	≤ 24 mg/L
gentamicin (pre + 1h post if on multiple doses)	2h	1d	See Micro. dept. guidance
lamotrigine	24 - 34h	7d	mono Px ≤ 15 mg/L multi Px ≤ 10
levetiracetam	7h	2d	6 - 20 mg/L
lithium - prophylaxis - acute mania	18 - 36h		0.4 - 0.8 mmol/L 0.8 - 1.2
mycophenolate	17h	4d	Depends on use
olanzapine			20 - 40 ug/L
oxcarbazepine (monohydroxycarbamazepine)	19h	4d	15 - 35 mg/L
paracetamol	1-4h		See BNF mg/L
phenobarbitone	child - 70h adult - 100h	child - 12d adult - 20d	neonate ≤ 30 mg/L adult ≤ 40
phenytoin	13 - 46h	14d	neonate 6-14 mg/L adult 10-20
pregabalin	6h	2d	See comment on report
primidone	8h	2d (see phenobarb.)	0 - 11 mg/L
sirolimus (rapamycin)	60h	10d	5 - 15 mg/L
tacrolimus (FK506)	11h	2d	Depends on use ug/L
theophylline	24h neonates 4h children 9h nonsmokers 4h smokers	5d 1 - 2d 2d 1 - 2d	10 - 20 mg/L
thioganine nucleotides	1w	4w	235 – 450 pmol/8x10^8 cells
thyroxine	7d	5w	TSH in ref. range
tiagabine	8h (3h, enz. induct)	3d	
tobramycin (pre + 1h post if on multiple doses)	2 - 3h	1d	See Micro. dept. guidance
topiramate	20 - 30h	1w	5 - 20 mg/L
valproate	12h	3d	50 - 100 mg/L
vancomycin (pre)	4 - 11h	3d	See Micro. dept. guidance
vigabatrin	6 - 8h	10d	5 - 35 mg/L
zonisamide	65h	2w	15 - 40 mg/L

TEXT on TDM.

Notes on TDM

- Assay is needed only for drugs with particular problems:
  toxicity eg. gent.
  narrow therapeutic range eg. Li
  variable absorption or clearance ie. dose does not reflect tissue conc. eg. digoxin, cyclosporin, phenytoin
- **Sample just before the next dose** to avoid false results in the distribution phase'
- **Sample not less than 5 plasma half-lives** after starting or changing dose ie before equilibration. Sampling before this is best avoided, unless **toxicity** is in question.
- **Even if serum levels correlate poorly** with clinical effect eg. valproate & vigabatrin, TDM may help if
  - compliance is doubted
  - there is clinical evidence of toxicity
  - there is unexpected loss of clinical effect or difficulty in establishing it.

HAEMOLYSIS

See comments & potassium.

Haemolysis causes enough interference in some assays to make the results unreliable. All requests & specimens received in the lab. are booked into the computer, centrifuged & placed on the analyser. **This machine (not a human) quantitates haemolysis** & blocks tests according to their individual vulnerability to interference from Hb & other intracellular contents eg. potassium.
BEWARE. A ward machine will give you a K result, haemolysed or not ie. you will not know the error.

Minimising haemolysis

- Ensure **free blood flow** ie. laminar flow. Turbulence is traumatic.
- **Gently** draw blood through cannulae.
- **Avoid heel-pricks** for large blood volumes.
- If a syringe is used, **gently** run blood down the inside wall of the sample tube.
- **Never** eject blood through a needle - remove it.
- **Never** shake, heat or freeze samples. Room temp. is best for routine tests.

ACUTE PHASE RESPONSE

See α1AT, albumin, C1INH, C3, C4, caeruloplasmin, chol. CRP, Fe, FT3, FT4, in-vivo influences, TIBC, TSH & Zn.

Reference ranges usually relate to healthy unstressed people. Illness not only perturbs specific analytes eg. cardiac troponin, but the stress & inflammation non-specifically change many factors to redirect the metabolic economy to recovery. This is termed the *acute phase response*. Within a day of the stimulus to inflammation, serum levels change eg. :

Levels ↓
albumin
cholesterol
iron
transferrin
selenium
zinc

Levels ↑
CRP
ferritin
α1-antitrypsin
haptoglobin
hemopexin
caeruloplasmin
α1-antichymotrypsin

} Results outside the reference range in health can be an appropriate response & are not automatically "abnormal" & in need of correction.

Sick-euthyroidism

- Related changes occur in thyroid physiology & hormone binding & are termed sick-euthyroidism**:**
- ↓ or normal TSH (sometimes transient ↑ at the start of inflammation.
- ↓ FT4 (sometimes transient ↑ on recovery).
- ↓ FT3
- Common conditions eg. subclinical 1° hypothyroidism & drug-effects, can interact to give odd TFTs.

Opportunistic screening eg. TFTs, lipids, iron & ferritin tests
DO NOT SCREEN 1d – 3m after the start of acute illness unless there is clinical evidence of abnormality, because acute & unrepresentative changes can occur, as above.

AKT

1956 – 2004

Turning a blind-eye to wickedness was not Andrew's way.

IN VIVO INFLUENCES ON RESULTS apart from illness.

See acute phase response & drugs & TFTs.

Results may directly reflect disease eg. ↑ serum CK in MI & ↑ prolactin in prolactinoma, or reflect physiological & pharmacological factors unrelated to disease eg. ↑ CK after exercise & ↑ prolactin with pregnancy & antipsychotic drugs. Give clinical details on the request form so that results can be more accurately commented on & necessary additional tests, added to the same specimen to minimise delay.

Influence	Examples of tests affected
gender	obvious hormones & many other tests eg. ACR, ALT, FT4, FT3, TSH, Fe satn, PO_4, CK.
menstrual cycle	FSH, LH, oestradiol, progesterone (day 21 level reflects ovulation/luteation). Androgens ↑ post ovulation (17OH progesterone, androstenedione, DHEAS, testosterone). Assay in follicular phase.
pregnancy	↓ FSH, ↓ LH, ↑ hCG, ↑ oestradiol, ↑ prolactin, ↓ FT4, ↓ FT3 & ↓ albumin…..
breast feeding	↑↑ prolactin.
age	many ref. ranges are age related eg. ALP, AFP, Ca, PO_4, TSH, FT4, FT3, oligoclonal SPE.
race	eGFR, CK are higher in black-americans & afro-carribeans
time of day	many analytes show a diurnal rhythm eg. serum cortisol, testosterone, PO_4 & urine ACR.
fasting	↑unconjugated bilirubin in Gilbert's syn. (also ↑ by tiredness, inflammation etc.) Fasting >16h becomes starvation eg. ↓ glucose, ↑ FFA, ↑ ketones, ↑ cortisol.
feeding	serum creatinine & urea ↑ after a meat-feast, even in health. PO_4 ↓ after CHO intake. There is a postprandial ↑ in Tg, cholesterol (rather less), glucose, B12 & gut hormones.
type of food	↑ 5HIAA from 5HT in nuts etc. ↑ Normetadrenaline, metadrenaline & methoxytyramine from metabolism of catecholamines in bananas & nuts. See "feeding". Restricted diets may cause false results: see amino acids, urine reducing substances & GTT.
exercise	zealous or unaccustomed exercise: can ↑ CK. FSH, LH & oestradiol may ↓ in women.
missing limbs	serum creatinine can ↓ & eGFR ↑misleadingly due to the lower muscle mass.
body-building	serum creatinine ↑ & eGFR falls misleadingly with ↑ muscle mass (& diet). Anaboloid abuse can cause endocrinopathy eg. hypogonadism in males & virilisation of females.
debilitation/↓Wt	↑cortisol, ↓ FSH, ↓ LH & ↓ oestradiol in women. Hypogonadotropic hypogonadism occurs in men too, but is less.
stress	↑ cortisol & ↓ dex. suppression in chronic stress eg. obesity, alcoholism & depression.
stimulants	false +ve "VMA" & HIAA results occur with ↑catecholamine & serotonin secretion due to tea, coffee, smoking & exercise. GTT results can ↑ too.
smoking	↑ serum PLAP, CEA & carboxy-Hb. See "stimulants" above. Do not smoke in a GTT.
inflammation	see acute phase response & "debilitation" above.
drugs *Please give details on the request form.*	• **TFTs** are affected by drugs eg. Li, amiodarone, NSAIDs, frusemide, anticonvulsants, glucocorticoids, dopaminergic drugs.
	• **TFTs** can be ambiguous on T4 & T3 replacement, especially with erratic compliance. Please write **"on T4"** or **"on T3"** on the request form to avoid *wasted resources & time*.
	• **renin & aldosterone** show major & type-specific changes with antihypertensives. **Give drug details** to maximise the info. from these costly reference centre tests.
	• **cortisol** is falsely ↑ by prednisolone which cross-reacts in the assay. Dexamethasone is not detected but it physiologically ↓ cortisol secretion (ONDEX) & the result.
	• **potassium** ↑ by ACEIs, ARBs, renin inhibitors, NSAIDs, β-blockers, K-sparing diuretics. ↓ by diuretics, corticosteroids, insulin, β-stimulants, licorice.
	• take **therapeutic drug-monitoring** samples **just before a dose** to avoid falsely ↑results during the distribution phase. Even drugs with a long half-life have one eg. thyroxine.
	• **gastrin** ↑↑ with PPIs & H2RBs. Stop them 14d & 3d before sampling, respectively.

CANNED COMMENTS ADDED TO RESULTS

See canned comments continued, haemolysis & Therapeutic Drug Monitoring

These are standardised comments which can be appended to results in order to highlight common causes & useful additional tests. Some or all suggestions may already have been executed & the diagnosis made, but this may be unclear to the lab. due to limited clinical details & time.

code	Analyte • Threshold for commenting • Comment.
hi amy	**Amylase >200 U/L. ?** pancreatitis • parotitis • bowel obstruction • neoplasia (esp. bronchus & ovary). *Suggested assays* ALP, ALT, bili., Ca, GGT, glucose, iron satn, PO_4 & triglyceride
hi Ca	**Calcium (total) >2.60 mmol/L. ?** ↑ albumin (see adjusted Ca) • 1° hyperparathyroidism • neoplasia • sarcoid • vit D XS • milk-alkali syn • .hypocalciuria (assay 24h urine Ca). *Suggested assays* ACE, bicarbonate, creatinine, K, Na, PO_4, PTH, urea & vit D
lo Ca	**Calcium (total) <2.15 mmol/L. ?** EDTA from FBC tube • ↓Mg • ↓vit D • ↓PTH • drugs • alcoholism • CRF. *Suggested assays* coeliac serology, creatinine, K, Mg, Na, PO_4, PTH, urea & vit D
hi cort	**Cortisol >1000nmol/L. ?** stress (Wt. loss, obesity, depression, alcoholism) • hydrocortisone • prednisolone • Cushing's syn • .hyperthyroidism. *Suggest* an overnight dexamethasone suppression test.
lo cort	**Cortisol <100nmol/L.** Caution, spot results can be low even in health, esp. late in the day. *Suggest* Synacthen test. In suspected Addisonian crisis, take blood for cortisol then give hydrocortisone.
hi gent	**Gentamicin >5.0 mg/L pre-dose.** See Microbiology dept. guidelines. ? sample time, dose & renal function. *Suggested assays* creat, K, Na, & urea
hi GGT	**GGT men >49, women >32 IU/L.** Induced by drugs eg. ethanol & phenytoin • cholestasis of any cause (ALP is frequently ↑ too). *Suggested assays* ALP, ALT, bilirubin, CK, ferritin, iron satn. & mcv
lo gluc	**Glucose** Hypoglycaemia = **≤2.5 (adults)**, **≤2.0 (children)** mmol/L. Insulin & C peptide will be assayed if an appropriate sample is provided. ?cause. Contact lab. if assistance is required with further tests.
hi Li	**Lithium >1.0 mmol/L. ?** sample timing • dose • renal function. *Suggested assays* creatinine, K, Na, TSH & urea
hi Mg	**Magnesium >1.0 mmol/L. ?** CRF • ARF • Addison's dis., • hypoaldosteronism • hypothyroidism • tissue trauma. *Suggested assays* aldosterone, cortisol, creatinine, K, Na, renin, TSH, urea
lo Mg	**Magnesium <0.60 mmol/L. ?** malabsorption • ↑losses • alcoholism • acute pancreatitis • pregnancy • hyperaldosteronism • ↓PTH • ↑Ca • DKA • SIADH • lactation. ↓Mg can cause ↓K & ↓Ca. *Suggest assays:* aldosterone, ALT, ALP, amylase, bili., Ca, creat., GGT, glucose, urea, K, Na, PO_4, PTH & renin
S osmo	**Osmolality serum.** Assay spot urine osmo & Na conc. too, for more info. Urine/serum osmo. ratio is normally 1.0 – 3.0. In DI, can be as low as 0.2-0.7. In polydipsia, the ratio rises with H_2O restriction.
U osmo	**Osmolality urine**. An EMSU osmo. >500 mmol/kg, after avoiding drink from 10pm the night before, excludes DI. See Na (urine).

Continued…….

Canned comments continued

hi PO_4	**Phosphate >1.5 mmol/L. ?** old sample • CRF • ARF • bone neoplasia • tumour lysis • vit D toxicity • ↓PTH • acromegaly. *Suggested assays* Ca, creat., IGF1, K, Na, PO_4, PTH, urate, urea & vit. D
lo PO_4	**Phosphate <0.9 mmol/L. ?** ethanol • time of day (↓ pm, ↑late am.) • post CHO-rich meal • glucose ivi • exogenous insulin • malnutrition • malabsorption • vit D deficiency • hyperPTH • renal tubular defects. *Suggested assays* Ca, creat., K, Na, PO_4, PTH urate, urea & vit. D.
hi K	**Potassium >5.5 mmol/L. ?** K-EDTA contamination from FBC tube • haemolysis • heel-prick • ++ fist-clenching • high platelet/wbc count • drugs eg. ACEIs, ARBs & NSAIDs • ↓GFR • ivi • acidaemia • Addison's. *Assay* aldosterone, bicarb., cortisol, creat., fbc, glucose, K, Na, urea & renin & if cause unclear
lo K	**Potassium <2.5 mmol/L. ?** drugs eg. diuretics, insulin & salbutamol • ivi. • ↓intake • ↑losses • mineralocorticoid XS • renal dysfunction • liquorice • alkalaemia • hypomagnesaemia. *Suggested assays* aldosterone, bicarb., Ca, cortisol, glucose, K, Mg, Na, PO_4, renin & urea.
hi Na	**Sodium >150 mmol/L. ?** Fluid & elec. balance • renal function • ivi • drugs. If cause unclear, *clinically assess ECF vol.* & relate the physiological aims predicted from this to osmo. & Na conc. of spot urine. Are your ideas confirmed?
lo Na	**Sodium <125 mmol/L. ?** fluid & elec. balance • ivi • renal function • Addison's • drugs • H_2O retaining states eg. post-op., oedema, ascites • SIADH. If cause is unclear, *clinically assess ECF vol*. & relate the physiological aims predicted from this to osmo. & Na conc. of spot urine. Are your ideas confirmed?
hi TDM	**Therapeutic drug level > upper ref. limit. ?** sample timing • dose • other drugs *Suggest assays* related to clearance eg. ALP, ALT, bicarb., bilirubin, creatinine, K, Na & urea
hi Vanc	**Vancomycin >15.0 mg/L pre-dose. ?** sample timing • dose • renal function. *Suggested assays* creatinine, potassium, sodium & urea. See Microbiology dept. guidelines.

A – Z of tests **Layout of A – Z**

See sample tubes, volumes & profiles & outline of Chemical Pathology.

A	**B**	**C**	**D**	**E**
F	**G**	**H**	**I**	**K**
L	**M**	**N**	**O**	**P**
Q	**R**	**S**	**T**	**U**
V	**W**	**X**	**Y**	**Z**

A – Z top

name

return hyperlink

Stability at 4°C ie. the latest "add-on"

min. blood vol.

sample tube

Test frequency. ***daily***: run at ESH routinely & urgently. ***sent***: sent away, result back in 1-4w

nature of ref. limits

units

FSH (Follicle Stimulating Hormone) 2d 1.5mL 

daily (5^{th} - 95^{th} *centile*)

See oestradiol & progesterone

women	follicular phase	3.5 - 12.5 IU/L
	mid-cycle peak	4.7 - 21.5
	luteal phase	1.7 - 7.7
	post-menopause	25.8 - 134.8
men	20 - 60y	1.5 - 12.4
Roche: insert V15		

hyperlinks to related matters

gender, age & physiology related categories

source of data

Reference data

Use – Clinical applications

Test – Sampling conditions, protocol etc.

Background – Basic science, clinical features etc.

Causes of ↑ & ↓ – Lists of influences on results (not necessarily all).

A

α1 antitrypsin (α1AT) faecal 0d 4mL

Sent

≤ 0.48 mg/g wet weight
Sheffield Protein Reference Unit

See albumin & A1AT (serum).

- **MUST** have its **own sample**.
- **Send promptly**
- **MUST be blood-free** (causes false +ve).

Use

- Ix & monitoring **protein losing enteropathy**, especially in children.
- **Stools + ve for blood** will not be tested.

Background

- ↓ functional or mechanical **integrity of the intestinal mucosa** causes loss of plasma proteins into the lumen.
- Degradation, especially by bacteria, impairs their use as markers of **protein losing enteropathy** in faeces.
- However, **A1AT** **resists degradation** more than other plasma proteins eg. antichymotrypsin.

Interpretation

- False **+ ves** eg. from g.i. bleeding
- False **– ves** eg. from **sample degradation**, congenital **α1AT deficiency.**

α_1 antitrypsin (α1AT) serum 3d 4mL

Sent

See A1AT (faeces), acute phase response & protein electro.

Use Ix emphysema below age 50y & hepatic cirrhosis.

	(5^{th} - 95^{th} cent.)
Birth	0.9 - 2.2 g/L
6 m	0.8 - 1.8
1 y	1.1 - 2.0
5 y	1.1 - 2.2
10 y	1.4 - 2.3
15 y	1.2 - 2.0
Adult	1.1 - 2.1
Sheffield Protein Reference Unit	

Background

- A 54kDa **glycoprotein** (plasma half-life 4d) secreted by the **liver**.
- Inhibits neutrophil **elastase** & macrophage **lysosomal proteases** to limit damage to healthy tissues during inflammation.
- The **alpha 1 band** (just behind albumin) in serum protein electrophoresis is mostly α1AT ie. deficiency is sometimes found incidentally.
- 1:2000 UK residents have severe deficiency (< 0.6 g/L). Can present as**:**
  COPD/emphysema before age 50y
  Liver disease
  - neonatal cholestasis
  - progressive juvenile cirrhosis
  - in adults as unexplained micronodular cirrhosis & necrotizing angiitis.
- 1:20 people are **heterozygous** for a mutant allele. Even with complete loss caused by a null form, the wild type allele provides sufficient function to be illness-free if risks are avoided eg. **smoking**.
- **Phenotyping** (elecrophoresis) to identify the PI (protease inhibitor) forms present, is **performed automatically** if serum α1AT level is **below the reference median**.
- **There are over 60 known α1AT (PI) variants** (named after their electrophoretic position or the town where they were discovered), with functions from nil to normal.
- The **relatives** of someone with deficiency need to be tested too (spouse, siblings, children & parents).

Causes of ↓

- congenital α1AT deficiency • cachexia • nephrotic syndrome • severe liver disease

Causes of ↑ Levels in carriers (0.6-1.4 g/L) can ↑ to the ref. range ie. postpone sampling in**:**

- acute phase response • chronic inflammation • ↑ oestrogen eg. HRT, OCP
- malignancy • pregnancy

acetylcholine receptor Ab 3d 2mL

Sent

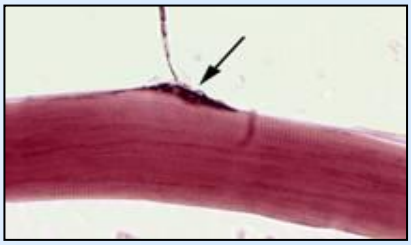
See cholinesterase (serum).

< 0.2 nmol/L + interpretation
Sheffield Protein Reference Unit

Use

- Ix of **myasthaenia gravis**.
  Correlation of Ab levels & type with disease activity & clinical presentation**, is weak.**

Background



- Antibodies (mostly type **IgG)** to acetylcholine receptors in skeletal muscle motor endplates.
- **Present in > 80% of patients** with myasthaenia gravis, but not in patients with the congenital form.

There are **3 types of Ab:**

1. **Blocking** Ab to the acetylcholine binding site.
2. **Binding** Ab to receptor-epitopes distinct from the acetylcholine binding site.
3. **Modulating** Ab which increase receptor degradation.

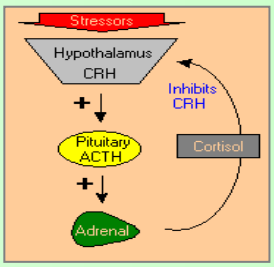
ACTH (adrenocorticotropic hormone) 0h 2mL

Sent

7 – 62 ng/L
Croydon Unversity Hospital

See androstenedione, CgA, cortisol, 5d dex. suppr. test, DHEAS, ONDEX & SST.

- **Needs its own tube**
- **Keep on ice**, get to lab. in 30 min.
- **Minimise venepuncture-stress.**



Uses

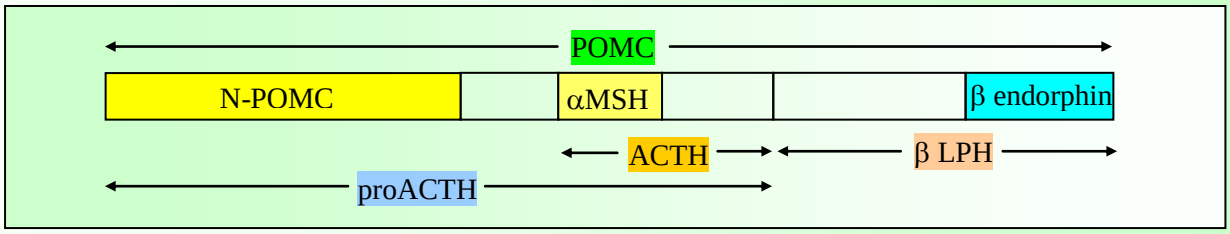
- Ix **cause of hyper** or **hypocortisolism** after presence established with cortisol assays.

Interpretation

- ACTH **levels overlap** in health & states of hyper & hyposecretion because of the pulsatile secretion & dynamic physiology ie. interpret results carefully & in the light of clinical details & prevailing cortisol levels.
- **Hypercortisolism**. If physiology is controlled with dexamethasone, ACTH assays can indicate the **cause** eg. Cushing's disease *vs.* ectopic ACTH syn. See **5 day dex suppr** test.
- **Primary hypocortisolism** eg. Addison's disease: plasma ACTH > 100 ng/L at **9am**.
- **Secondary .. ..** eg. pituitary or hypothalamic deficiency: plasma ACTH is usually low, but can be in ref. range (Arlt 2003). Low cortisol is the clue to the real meaning, hence the **primary importance of cortisol assays**.

Background

- ACTH (39 aa) is cleaved from a **precursor** (pro-opiomelanocortin, **POMC,** 241 aa), which is also the precursor for other signalling molecules eg. β-endorphin. The peptide formed & secreted by a tissue is determined by the particular proteases expressed within the secretory pathway (Jackson 2003 & 2009).



- In **ectopic ACTH** syndrome a wide range of ACTH levels & clinical & biochemical phenomena occur, which are unlike Cushing's syndrome eg. hypokalaemic alkalosis. One reason is the **disorded processing of POMC** with ↑ secretion of ACTH precursors, which may have ↓ or alternative bioactivity, despite ACTH immunoreactivity eg. POMC, proACTH & N-terminal POMC. Assays which are very specific for ACTH may give relatively low results & paradoxically be less sensitive for detecting "ectopic ACTH" (Crosby 1988). Precursor levels have even been reported to correlate with cortisol levels in the ectopic ACTH syndrome, better than those of ACTH itself, hence the term "**ectopic ACTH precursor syndrome**" (Stewart 1994).

- ACTH stimulates melanocortin type 2 receptors (**MC2R**) on adrenocortical cells in 1) the **zona fasciculata**, causing secretion of cortisol & 2) the **zona reticularis**, causing release of androgen intermediates eg. androstendione & dehydroepiandrosterone (**DHEA**) & its metabolite DHEA sulphate (Cone 1996) ie. **adrenal androgens** show **pulsatility** & a **diurnal rhythm** like plasma cortisol, except DHEAS which has too long a half life.

- Despite this textbook view, POMC, ACTH & the MC2R are also expressed, albeit at low levels, by many **non-endocrine tissues** eg. lymphocytes & keratinocytes. Is stress the common factor?

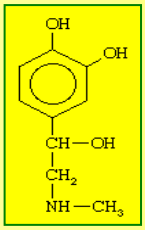
adrenaline 3d acidified 24h urine

Sent

See VMA.

- **Plain bottle**
- **Acid is added on return to the lab**.

Children only, 20mL urine



< 100 nmol/24h
St. Helier Hospital

AFP (α-fetoprotein) 3d 1.5mL See profiles

Daily

See ALT, CA125, CA153, CA199, CEA, hCG, **MRI** & PLAP.

- **In germ cell neoplasia, check hCG too.**
- **NOT FOR** Down's screening.

Use

- **Tumour marker**: hepatocellular carcinoma (HCC), non-seminoma germ cell tumours & hepatoblastomas
- **Acute response to Px** eg. total excision
- **Screening** hi-risk cases for HCC eg. hep B cirrhosis.
- Part of **antenatal screen** for Down syn. & NTDs.

Age	Male	Female
1d - 30d	< 13600 kU/L	< 15700 kU/L
1m - 1y	< 23.2	< 63.9
2y - 3y	< 6.6	< 9.1
4y - 6y	< 4.6	< 3.5
7y - 12y	< 3.1	< 3.1
13y - 18y	< 3.2	< 3.2 (non-preg)
Adult	< 5.8	< 5.8
(*95th centiles*)		
Roche Cobas AFP method sheet V5 & Roche Reference Ranges for Adults & Children, 2004.		

Background

- 70 kDa **glycoprotein** functionally similar to albumin.
- Synthesised by **fetal** liver, yolk sac & gi tract from 10th w of gestation (10/40).
- Serum AFP **peaks at 20/40** then ↓ to term.
- **Post-natally**, serum-AFP ↓, **half-life** of 5 – 7d. Can take up to a year to reach basal levels.
- **Post-resection** of HCC (complete), AFP plasma **half-life** = 3.5 – 4d. Longer if incomplete.
- **Preterm babies** have **higher & more persistent** levels consistent with their gestation.
- Levels ↑ slightly after age 50y.

Causes of ↑

- **Pregnancy** – higher if multiple
- Hepatic **regeneration** eg. with hepatitis, cirrhosis & trauma
- Primary **hepatocellular carcinoma** (AFP ↑ in 80% of cases), levels may be v. high
- **Hepatoblastoma** (AFP 500 - 10,000 kU/L in nearly 100% of cases)
- **Liver mets.** from any primary, but usually AFP is < 400 kU/L
- Gonadal & extragonadal **yolk sac or endodermal sinus tumours** (teratomas, non-seminomatous germ cell tumours)
- **Neonatal hepatitis** (biliary atresia, cholestasis & α1AT deficiency, rarely ↑ AFP)
- Chemotherapy can ↑ AFP for months, notably **cis-platinum**
- **Ataxia telangiectasia**
- herditary **tyrosinaemia**
- Upper **g.i. carcinoma**
- Open **neural tube** defects

Causes of ↓

- Pregnancy with a **fetus with Down's** syn.

.

albumin 3d See profiles

See acute phase, AFP, ALT, AST, bilirubin, Ca (total), Ca (adj.), **MRI**, SPE & total protein.

Daily

0 - 4d	28 - 44 g/L
4d - 14y	38 - 54
14y - 18y	32 - 45
Adult	35 - 52
Roche Cobas ALB2 method sheet V4	

Background

- A 66 kDa non-glycated peptide synthesised in the **liver**.
- The **ratio of entry to exit** of albumin from the circulation determines the plasma conc.
- **Hepatic function is not a major determinant** until it is markedly impaired.
- Similarly, **nutrition**.
- Albumin provides **amino acids**, **oncotic pressure & storage & transport** of hormones, metals, drugs, fatty-acids... ↓ affects the toxicity, action & interactions of bound substances eg. bilirubin, warfarin, fatty acids.
- Serum **total Ca** changes with albumin conc., because *ca.* half of Ca is albumin bound. See adjusted calcium.
- **Analbuminaemia** (congenital albumin deficiency) causes only mild dependent oedema, despite Prof. **Starling.**

Causes of ↑

- **Venepuncture cuff** over-use.
- **Dehydration**.

Causes of ↓

- ↑ **trans-capillary escape** due to the acute phase response, even though albumin **synthesis** may ↑.
- ↑ **albumin loss** eg.
  - nephrosis
  - protein losing enteropathy. See A1AT (faecal).
  - burns (also trans-capillary escape, catabolism & impaired feeding).
- **Nutrition**: use albumin cautiously as a marker. Malnutrition promotes hypoalbuminaemia, but it commonly coexists with other factors which may be quantitatively more important eg. the acute phase response (see above).
- ↑ **catabolism** eg.
  - burns
  - fever
  - malignancy
  - thyrotoxicosis
  - Cushing's syndrome
- **DNA variants**.
- **Pregnancy**
- **Hepatic failure** – but this may co-exist with other causes eg. acute phase response.

albumin/creatinine ratio (ACR) 3d 5mL **(EMU)**  Daily

See creatinine, eGFR, glucose, HbA1c, protein/creatinine ratio, protein (24h urine) & **MRI**.

- **Early morning urine** (EMU) is best: – fewer false + ves from orthostatic proteinuria.
  – results are more precise because urine is more concentrated.

Background

- ↑ urinary albumin (66kDa, relatively small & only just too big for "normal" glomerular "pores") is a marker of **early nephropathy** when intervention eg. strict control of BP, can slow or reverse CKD & ↓ CVD risk.
- **Microalbuminuria** is proteinuria in which urine albumin excretion exceeds the 95th centile level in health, but is below the detection limit of ordinary "dip stix" ie. the albumin loss is **30 – 300 mg/24h** or **20 – 200 ug/min**. These *roughly* correspond to concentrations of 20 – 200 mg/L of albumin.
- **24h urine** collections for measuring microalbuminuria are inconvenient & vulnerable to collection-errors.
- **Spot urine** samples are easy to collect, but dilution affects concentration. This is adjusted for by presenting the mass of albumin as a **ratio to creatinine** (ACR) rather than water. This works because creatinine is produced by muscles at a constant rate (see creatinine) ie. the urinary creatinine mass is a marker of time.

Causes of ↑ ACR Women have a higher ACR than men – lower muscle mass = less creatinine excretion.
• nephropathy • UTI • ↑BP • fever • exercise • pregnancy • standing • menstruation

DIABETIC PATIENTS

- For **monitoring** onset of nephropathy & guiding treatment.
- An unexpectedly ↑ result in absence of proteinuria or UTI, is **confirmed** if 1 of up to 2 further samples within 2 months of the first result, is + ve.

Women	0 – 3.5 mg/mmol
Men	0 – 2.5
NICE CG15 & CG87	

NON-DIABETIC PATIENTS AT RISK OF CKD

- For **monitoring** onset of nephropathy & guiding treatment.
- Initial ACR 30 – 70 mg/mmol: confirm in EMU (**higher action-threshold than in diabetes**) NICE CG73.
- .. .. ≥ 70 mg/mmol or PCR is ≥ 100 mg/mmol, **confirmation is unnecessary**.
- **Approx. equivalent** ACR, Protein Creatinine Ratio & 24h urine protein results:

	ACR mg/mmol	**PCR** mg/mmol	**24h urine protein** g/24h
Normal	Women 0 – 3.5 Men 0 – 2.5	0 – 45	≤ 0.14
Clin. sig. proteinuria	30 – 70	approx. 50 – 100	approx. 0.5 – 1.0
Heavy ..	≥ 70	.. ≥ 100	.. ≥ 1.0
	NICE CG 73		

BACKGROUND

NICE in 2008 (CG73) up-dated its advice on the identification & management of chronic kidney disease (**CKD**) in adults to include the use of **ACR in non-diabetic patients too for the detection of early proteinuria** in preference to 24h urinary protein quantitation, protein/creatinine ratio & dip-sticks, because ACR has better **sensitivity**, repeatability, ease of use & ↓ influence by dilution. PCR can be used to monitor & quantify *known* proteinuria.

ACR & eGFR are recommended for screening all adults at risk of CKD due to:

- eGFR < 60 mL/min/ 1.73 m^2
- hypertension
- CVD: - ischaemic heart disease
  - peripheral vascular disease
  - cerebrovascular disease
  - chronic heart failure
- opportunistic detection of haematuria.
- structural renal tract dis., multiple renal calculi or prostatic hypertrophy
- multisystem diseases which can affect the kidney eg. SLE
- FH of stage 5 CKD or heritable kidney disease.

Test-intervals & responses to results. See NICE CG73.
- annual ACR + eGFR to screen people at risk of CKD.
- confirm 1st ACR if ≥ 30 mg/mmol.
- 6 or 3 monthly ACR in established CKD depending on stage.

aldosterone 3d 4mL

Sent

See ACE, CgA, comments, cortisol, HIAA, K, Na, 17-OHP, renin & VMA.

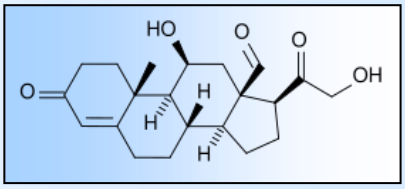
Adult recumbent	100 – 500 pmol/L
.. ambulant	600 – 1200
University College London Hospital	

- **Needs its own sample.**
- **Only renin assay can share.**

Use

- Ix of **hyper** & **hypoaldosteronism** (not Addison's dis.) eg. abnormalities of BP, serum Na & serum K.

Test



- **Measure renin at the same time** to get the full-picture.
- Specimen must reach lab. **within 4h**
- Send patient to **Crawley** or **ESH phlebotomy** clinic if this is difficult.
- **Give drug details on the request form** to get better comments.
- **Ideally**, take samples 1) before patient has even sat up in bed in am.
  2) & 30 min. after getting up.
- **OPD/GP surgery.** Samples for renin & aldosterone after 20 min. rest in the waiting room, can exclude hypo & hyperaldosteronism in most cases.

Background

- Secreted by the **zona glomerulosa** of the adrenal cortex, in response to **angiotensin II**.
- Acts on the **distal renal tubule** to ↑ **uptake of Na** from tubular fluid in **exchange for K & H^+**.
- **Gut & sweat glands** also have aldosterone receptors, but the clinical significance is little.
- Receptors have **low specificity** & would be **stimulated by cortisol** (plasma conc. 1000x that of aldosterone) if it were not for *11β hydroxysteroid dehydrogenase type 2* at the receptor which blocks the action of cortisol by converting it to less active **cortisone**.
- **Liquorice** inhibits this enzyme, thus cortisol has a much ↑ mineralocorticoid-like effect.

Causes of ↑

- **Primary hyperaldosteronism** – adenoma (Conn's syn.) & hyperplasia. Renin ↓ & R/A ratio ↓ too.
- **2ndy .. ..** eg. CCF, diuretics, nephrosis, ascites, hypovolaemia, pregnancy, mid-late luteal phase. Renin ↑

Causes of ↓

- **Hypertensive** eg. CAH (11β-hydroxylase deficiency), exogenous mineralocorticoids/corticosteroids, liquorice.
- **Not hypertensive** eg. Addison's disease, hyporeninaemic hypoaldosteronism.

alkaline phosphatase (total) 3d See profiles

Daily

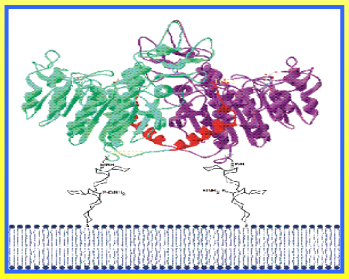
See ALP isoenzymes, ALT, AST, bilirubin, Ca, Ca adj, comments, GGT, **MRI**, PLAP, PO_4, PTH, vit D & Zn.

Use Ix of liver & bone pathology.

	Age	**ALP** (U/L)
Children	1d	< 250
	2d - 5d	< 231
	6d - 6m	< 449
	7m -1y	< 462
	1y - 3y	< 281
	4y - 6y	< 269
	7y -12y	< 300
	girls 13 - 17y	< 187
	boys 13 - 17y	< 390
Women		35 - 104
Men		40 - 129
Roche Cobas ALP2 method sheet V 3		

Background

- ALPs are **glycoprotein homodimeric enzymes**.
- Each chain is covalently linked by its C-terminus to a **GPI** anchor (**g**lycosyl**p**hosphotidyl**i**nositol) in the outer layer of the plasma membrane. See glycolipid.
- Assayed for 100y, but function remains unsettled.
- ALPs **hydrolyse monophosphate esters** under alkaline conditions eg. 1) phosphoethanolamine, 2) pyridoxal phosphate & 3) phosphorylated molecules prior to transport across membranes.
- **Transmembrane transport role**: ALP is abundant in biliary, gut, placental & renal tubular epithelium.
- **3 genes** & 3 types of ALP
  - **liver/bone/kidney**
  - **placental**
  - **intestinal**
- **Liver, bone & kidney** ALPs have **tissue specific differences in carbohydrate content**.
- **Hypophosphatasaemia** caused by quantitative mutations of the L/B/K ALP gene, is without apparent effect, despite the widespread expression of ALP, presumably because sufficient activity is retained to avoid disease. However, rare alleles exist which cause marked deficiency & skeletal, dental & neurological effects in children.



Causes of ↓

- EDTA contamination from FBC tube (↑ K, ↓ Ca too).
- Hypothyroidism.
- Congenital variant.
- Hypochondroplasia.

Causes of ↑

- Much commoner & listed elsewhere in terms of the ALP isoform involved.

ALP isoenzymes 3d 3mL Sent

See ALP (total), GGT & PLAP.

Total	**ALP**	30 - 126 U/L
Liver	**..**	0 - 60
Bone	**..**	0 - 70
Intestinal	**..**	0 - 20
Great Ormond Street Hospital		

Uses

- Ix unexplained **isolated ↑ALP** (normal GGT).
- **Tip** – measure GGT if an ↑ ALP is of unknown source. Both are induced by **cholestasis** of any cause eg. drugs, stones, cirrhosis. ↑ GGT suggests ↑ ALP is hepatic in origin.
- Cause of ↑ ALP usually found by clinical skill, LFT, GGT, U+E, bone profile & vit. D assay & imaging.

Bone ALP - from **osteoblasts**.

- **Causes of ↑:**

- Normal growth
- Fractures
- vit. D deficiency
- Paget's disease of bone
- Neoplastic infiltration of bone (myeloma usually does *not* unless fractures occur).

- PTH stimulates **osteoclasts** & only ↑ ALP when bone disease is gross or there is a fracture.

Liver ALP - from **biliary canaliculi** & **pericanalicular hepatocytes**.

- Hepatic ALP contributes just over **half of the adult total ALP level**. Less in the elderly & in pregnancy when bone & placental ALP ↑, respectively. Growing babies & children show a much ↑ bone component. Adult proportions of liver ALP are finally reached at about age 20y.
- **Causes of ↑** Induced by **cholestasis** of any cause:

- cholelithiasis
- cirrhosis
- drug effects
- hepatitis
- intrahepatic masses.

- In **intrahepatic cholestasis**, serum ALP (& GGT) may ↑, while bilirubin levels remain normal if sufficient canaliculi are open for bilirubin to be excreted.

Kidney ALP – high expression in **tubular epithelium**.

- No known correlation with disease.

Placental **ALP** (PLAP)

- **Causes of ↑:**

- pregnancy
- smoking
- some carcinomas eg. **seminoma**, carcinoma of **breast & ovary**.

Intestinal ALP

- **Causes of ↑:**

- postprandially in secretor + ve people of **blood groups B & O**
- **Transient hyperphosphatasaemia of infancy.** Serum total ALP 3-5,000 U/L for up to a few weeks. Mild g.i. & flu-like symptoms suggest a viral cause.

- Intestinal ALP is a minor part of total ALP & inconsistently related to disease.

ALP (placental) (PLAP) 3d 1.5mL

Sent

Non-smokers	≤ 0.5 U/L
Smokers	≤ 1.5
Sheffield Protein Reference Unit	

See AFP, ALP isoenzymes, ALP (total) & hCG.

- Give details of **recent smoking.**
- Results are **assay-dependent.**
- **DO NOT** compare results & reference values of different assays.

Use

- Monitoring gonadal & extragonadal **germ cell tumours** in combination with AFP & hCG assays.

Background

- 3 types of PLAP:

- **Placental**, secreted by **trophoblast** in 3rd trimester, can ↑ serum total ALP 1.5x the non-pregnant level (as high as 12x in some normal pregnancies). Disease related mutations are unknown.
- **Regan isoenzyme,** a placental-like ALP. Normally present at low levels in lung, cervix, ovary, testis & breast. Secretion ↑ by **smoking** (Mc laughlin et al 1984).
- **Nagao isoenzyme**, another placental-like ALP, expressed at low levels in normal lung, testis & cervix. ↑ in seminoma, dysgerminoma, lung & gynae. cancer, but assay is useful only in the 1st two.

Interpretation

- Serum **half-life = 3d**. A slower fall in levels after primary therapy suggests incomplete removal.
- PLAP is not ↑ in **non-malignant** testicular disease.
- ↑ in 55% of seminomas, 60% of dysgerminomas, but not usually in teratomas. 10% of cases, PLAP is sole marker.
- May ↑ in ovarian **carcinoma**, but no more useful than CA125.

ALT (alanine aminotransferase) 3d See profiles
See AFP, albumin, ALP, amino acids, AST, **MRI**, AST/ALT ratio, biliribin, comments, GGT, urea & vit. B6.

Daily

Women	≤ 33 U/L
Men	≤ 41
Roche Cobas ALT method sheet V3	

Background

- Aminotransferases eg. ALT & AST, catalyse the **transfer of amino groups** from amino acids (AA) to **α-keto acids** & the reverse ie. they are key players in metabolism eg. synthesis of proteins & AAs & their breakdown.
- The prefix **keto means** that the carbon of the carbonyl group (**C=O**) bonds to 2 other carbon atoms (in aldehydes the carbon of C=O is bonded to 1 or 2 hydrogen atoms). An α-keto acid is like an AA but the α-carbon forms a carbonyl (C=O) group rather than bond an NH_2 group & a hydrogen atom (see amino acids diagram).
- ALT & AST are "**spilt**" from injured cells eg. by trauma, viruses, toxins, drugs…ie. **not induced** like GGT
- ALT is **more liver-specific than AST**, but significant levels exist in:
  - kidneys
  - heart
  - skeletal muscle
  - pancreas
  - lungs

  } ie. **ALT can ↑ with injury to tissues other than liver**.

Causes of ↑ (1 – 3x)

***Rough* guidelines since ALT ↑ depends on disease-scale & natural history-stage.**

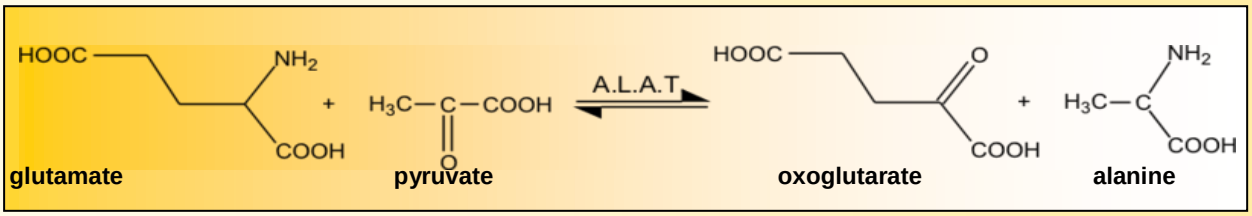
- drug side-effects
- disease/injury of non-hepatic tissues eg. heart, skeletal muscle, pancreas, lungs
- some of the causes immediately below:

Causes of ↑↑ (2 – 10x)

- drug side-FX
- CCF
- fatty liver
- ethanol
- cholestasis
- cirrhosis
- liver trauma
- chronic hepatitis
- glandular fever
- metabolic disorders.

Causes of ↑↑↑ (10 – 100x)

- viral & toxic acute hepatitis eg. paracetamol OD.
- circulatory collapse.



aluminium 3d 4mL

Sent

See Ca, creatinine, eGFR, PO_4, PTH & vit. D.

Use Ix aluminium intoxication.

Background

- **Inessential**, **ubiquitous** & can accumulate to **toxic** levels in patients with **CRF**, causing:
  - encephalopathy
  - vit. D resistant osteomalacia.
- Plasma levels & **intoxication much** ↓ by:
  - avoidance of Al based phosphate binders
  - dialysis systems with low Al content
  - reverse osmosis for purification of water
- **Circadian rhythm** - zenith 9 am, nadir 6 pm.
- **Transferrin** binds most circulating Al.
- **Bone** stores much Al, which can be released by sepsis.

Plasma Al (umol/L)	**Clinical effect**
< 0.37	Normal
0.37 – 2.22	Low risk of toxicity to all
2.22 – 3.70	Adults – excess Al *Children* – risk of toxicity
3.70 – 7.41	Adults – concern *Children* – high risk of toxicity
> 7.41	High risk to all
Royal Surrey County Hospital	

Causes of ↑

- **CRF** +/– haemo or peritoneal dialysis, Al based PO_4 binders, Al in water, long-term TPN, plasma exchange.
- **Peak** plasma level at **9 am**.
- **Sepsis**

amikacin 2d 1.5 mL

Sent

See gent., TDM, tobra. & vanc.

mg/L
Royal Brompton Hospital

Availability

- Get samples to the lab. **by these times** for same-day results. Late samples will wait until next day.
  - Mon – Fri **17.00** h
  - Sat + Sun **13.00** h

Contact Microbiology for advice on interpretation & patient management.
See IPCAS on the SASH intranet for sample timing, target levels, responses to results, doses etc.

amino acids (plasma) 1d 1.5mL
See ALT, amino acids (urine), ammonia, AST, carnitine, GAGs, homocystine, organic acids, orotic acid, urea, VLCFA & wbc enz.

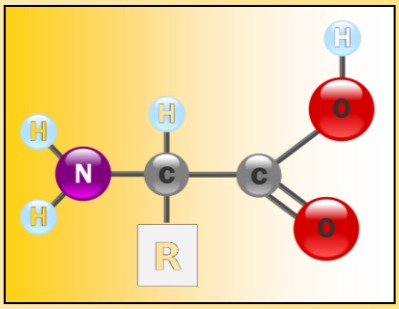
or


Sent

Refs & comment sent with result
Great Ormond Street Hospital

Use Ix inborn errors of metabolism (IEMs)

Getting the best from the test



- Sample during a **clinical episode**, ideally.
- No dietary restriction.
- **Please give details** of clinical features, food intake & sample timing, to aid interpretation of the results: plasma a.a. levels vary with:
  - age
  - chronic protein intake
  - fasting
  - feeding
  - non-specifically in response to systemic illness.

Background

- The **20** familiar genetically coded amino acids (AA) are **α-amino acids** with the general formula shown above, in which an **amino** & a **carboxyl** group are bonded to a common carbon atom (the **α carbon**) to which a side-chain (**R group**) is also covalently bonded. It is the structure & properties of this group which determine the nature of the AA eg. just a hydrogen as in glycine or a carboxylic acid as in aspartic acid.
- The α-amino group of one AA can react with the α-carboxyl group of another to form an amide (**peptide**) bond. Repetition of this process forms a **protein**.
- There are **additional amino acids** which are not encoded by codons:
- **Modifications** of α-AA in proteins eg. hydroxyproline in collagen.
- Intermediary **metabolites** eg. citrulline & ornithine (essentially α-AA with special R groups).
- **Non α-AA**: the amino group is not bonded to the α-carbon with the carboxyl & R-group as in α-AA eg. the neurotransmitter γ-aminobutyric acid (GABA). In this, the amino group is bonded to the 3rd in a chain of carbon atoms named α, β and γ.
- **Taurine** is often called an amino acid but strictly it is not, because sulphonic acid replaces the carboxyl.
- The **inter-connection of metabolism** of protein, carbohydrate & lipid means that even defects of enzymes not directly involved in AA metabolism, can affect AA plasma levels. Also, defective metabolism of one amino acid affects levels of others. Thus, **plasma AA assay involves** simultaneous measurement of a wide spectrum of amino acids, because the pattern of changes can be as important to diagnosis as the level of an individual one.

Non-IEM causes of changes ie. false – ves & + ves.

- Dietary restriction
- malnutrition
- sample degradation
- AA ivi.

amino acids (urine) 1d 5mL

Sent

Assays + interpretation
Great Ormond Street Hospital

See amino acids (plasma), cystine (urine), organic acids & vigabatrin.

- **Give drug details** on the request form - some drugs eg. ampicillin & vigabatrin give false + ves.
- **No dietary restriction**

Use For Ix of:

- defects of **intestinal** AA absorption
- .. .. **tubular** .. .. eg. Hartnup dis. • lysinuric protein intolerance • cystinuria • Fanconi syn.

PLASMA amino acid (AA) chromatography is the **preferred screen for IEM** (inborn errors of metabolism) which directly or indirectly involve AA eg. maple syrup urine disease & **organic acid** & **urea cycle** defects.

Background

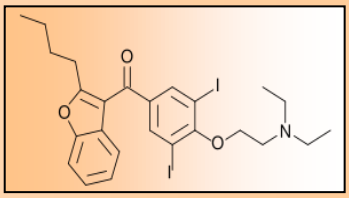
- AA cross plasma membranes via **specific transporter** proteins eg. in **cystinuria**, the mutation impairs a transporter protein for reuptake from glomerular filtrate of the basic AAs cystine, ornithine, arginine & lysine (**COAL**). In the **gut**, loss of function is incomplete because other transporters can substitute & prevent nutritional deficiency. Medical problems are limited to **nephrolithiasis** because of the high urinary conc. of cystine & its low solubility.
- Other transporters have **less redundancy** eg. in **Hartnup** disease, mutation of the gene for a **neutral amino acid transporter** in the gut & renal tubules, causes not only aminoaciduria but **malabsorption**, notably of tryptophan. This results in **niacin** deficiency (**pellagra** & neurological complications) but not renal disease because the unabsorbed aminoacids are soluble in urine (unlike cystine) & are just excreted.
- Urine amino acid **chromatography** shows characteristic patterns of unabsorbed amino acids in these diseases, but plasma amino acids should be examined at some point in order to exclude overflow aminoaciduria.

amiodarone 3d 1.5mL

Sent

0.5 – 2.0 mg/L
St Helier Hospital

See desethylamiodarone, drugs & TFTs & therapeutic drug monitoring (TDM).



Use

- **Confirming compliance**: a ratio of amiodarone to DEA concs. of > 3 suggests intake for only a few days. Levels are more equal on chronic therapy.

Background

- **Absorbed slowly** (3 – 7h).
- **Large distribution vol.** in fat, liver & lungs.
- **Long half-life** eg. **100d** on long term medication ie. its **effects persist for months**.
- **Serum levels correlate weakly with clinical phenomena**. This is partly related to measurement of levels before equilibration ie. 5 x half-life = 3 – 12 months.
- It has a major active metabolite, **desethylamiodarone** (**DEA**).
- The 2 iodine atoms per molecule give a **large iodine load** which can ↑ or ↓ thyroid hormone secretion.
- Amiodarone can also **↓ peripheral T4 to T3 conversion** which can cause normal or ↑ TSH, ↑ FT4 & ↓ FT3.
- TSH (& LFTs) should be **checked 6 monthly**.

ammonia 0d 1.5mL

Daily

Female	11 – 51 umol/L
Male	16 – 60
Roche Cobas NHL3 method sheet V8	

See amino acids, AST, ALT, carnitine, organic acids, orotic acid, urea, uric acid & wbc enzymes.

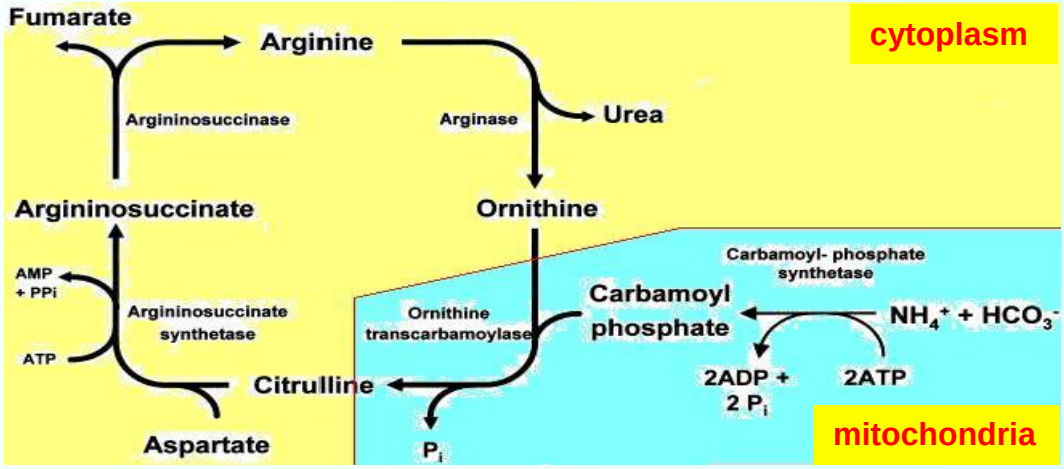
- **Send on ice** asap
- **Non-fasting** ideally: beware false – ves
- **Normal protein intake**
- **During a clinical episode,** if poss.

	Rough guide to interpretation
< 100 umol/L	Equivocal
> 100	Abnormal (may be seen in any fitting child)
> 200	Encephalopathy

Use Ix of hepatic failure • hypoglycaemia • IEM, especially urea cycle defects.

Background

- Ammonia (NH_3) is actually present as the **ammonium** ion (NH_4^+) at body pH.
- A by-product of **oxidative deamination** (esp. in muscle) & **bacterial metabolism** of nitrogenous substances in gut.
- **Detoxified** by synthesising the innocuous substance urea from it, especially in the liver:

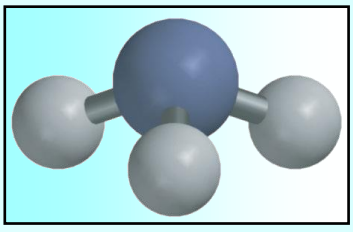


- Despite the apparent isolation of pathways affected by inborn errors of metabolism:
  - **amino acids** eg. lysinuric protein intolerance,
  - **fat oxidation & organic acids** eg. propionic acidaemia
  - the **urea cycle** eg. *ornithine carbamoyl transferase* deficiency,

  inter-connection of the pathways mean that not only can urea cycle defects can cause hyperammonaemia, but defects of carbohydrate, fat & amino acid metabolism can too ie. plasma ammonia assay is a useful **screen**.
- Patient should:
  - **not fast (**↑ ammonia may occur only after protein intake eg. citrullinuria).
  - have **normal protein intake**
  - ideally have a **clinical episode** when the sample is taken.

Causes of ↑: children need plasma amino acids • carnitines • urine organic acids • urine orotic acid assays too.

- **Haemolysis**
- ↓ **clearance** eg. due to:
  - urea cycle defects
  - inborn errors of amino acid & organic acid metabolism
  - fatty acid oxidation defects
  - portal/systemic shunting
  - Reye's syndrome
  - hepatic failure.
- **Valproate** Px.



amylase 3d 1.5mL

Daily

28 – 100 U/L
Roche Cobas AMYL method sheet V3

See Ca, comments, elastase & triglyceride.

Use • Ix of acute pancreatitis

Background

- **Hydrolyses** the bonds between adjacent glucose units in linear & branched chains eg. starch.
- **2 types** of amylase in plasma - different genes, alleles & glycosylation:
- **P type** from pancreatic acinar cells – mostly
- **S type** from salivary glands, tears, lungs, testes & fallopian tubes.
- Plasma **half-life** = 10h
- **Ca dependent** & inactivated by acid eg. stomach. Thus, result is lower in EDTA & citrated plasma.
- In **acute pancreatitis**, serum amylase typically ↑ 2 – 12h after symptom onset, peaks at 24h & lasts 3 – 7d.
- As **a marker** of acute pancreatitis (amylase ≥ 3x upper ref. limit), **more sensitive but less specific than lipase** ie. more false + ves. Overcome by clinical evidence & imaging (the gold standard). False – ves occur too.
- Height of ↑ correlates poorly with scale of pancreatitis.
- Persistent ↑ (several weeks) suggests: continuing inflammation, pseudocyst, pancreatic duct obstruction.

Causes of ↑

- race - africans & asians may have naturally ↑ S type level, causing ↑ total amylase in health
- acute pancreatitis
- trauma of pancreas
- cancer of pancreas, prostate, ovary, thyroid, bronchus
- pancreatic surgery
- chronic pancreatitis may ↑ serum amylase activity, but usually it is normal
- renal failure
- salivary disease: infection, trauma, irradiation, calculi
- mesenteric infarction
- biliary obstruction
- appendicitis
- pregnancy
- DKA
- drugs eg. opiates, diuretics, corticosteroids
- pulmonary inflammation
- cerebral trauma
- macroamylasaemia: biologically inconsequential aggregation of circulating amylase which causes false ↑ result. May affect up to 1.5% of hospital patients.

androstenedione 2d 1.5mL

See ACTH, 11-deoxycortisol, DHEAS, 17-OHP & testosterone.

Sent

Uses

- A **weak marker of CAH** (congenital adrenal hyperplasia) due to *21* & *11β-hydroxylase* deficiency (see 17OHP).
- 17-hydroxyprogesterone & 11-deoxycortisol, respectively, are superior.
- Ix of ↑ **serum testosterone** in women.
- Ix of ***17β-hydroxysteroid dehydrogenase*** (17 βHSD) deficiency. ♂ (XY) babies may have ♀ or incompletely male genitalia: testosterone synthesis is ↓ (see 17OHP). The ratio of serum androstenedione to testosterone is ↑ because they are the substrate & product of 17βHSD. Infants may need hCG stimulation to reveal excess androstenedione.

♀	**Adult**	postmenopause	2.0 - 12.0 nmol/L
		reproductive age	1.2 - 8.6
	Child	Tanner 1 <9.2y	0.28 - 1.74
		2 9.2 - 13.7y	1.46 - 3.48
		3 10.0 - 14.4y	2.78 - 6.61
		4 10.7 - 15.6y	2.68 - 7.83
		5 11.8 - 18.6y	2.78 - 8.35
♂	**Child**	Tanner 1 <9.8y	0.28 - 1.74
		2 9.8 - 14.5y	1.08 - 2.26
		3 10.7 - 15.4y	1.74 - 3.48
		4 11.8 - 16.2y	1.67 - 4.87
		5 12.8 - 17.3y	2.26 - 7.30
St Helier Hospital			

Background

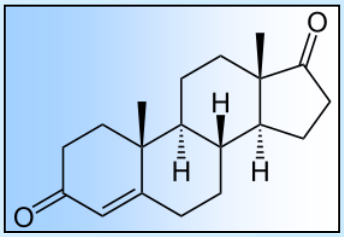
- Androstenedione is **a weak androgen** & **precursor to testosterone** & **oestradiol** synthesis in the adrenal cortex, ovary & adipose tissue. DIAGRAM.
- Serum androstenedione is approx. half **ovarian** & half **adrenocortical** in origin, in women of reproductive age.
- In contrast, the weak androgens dehydroepiandrosterone (**DHEA**) & dehydroepiandrosterone sulphate (**DHEAS**) come from only **the adrenalcortex** & are regulated by **ACTH**.

Causes of ↑

- PCOS (some cases)
- Congenital adrenal hyperplasia (CAH)
- Cushing's syn.
- Ectopic ACTH syn.
- Neoplasia/hyperplasia of ovary

Causes of ↓

- Adrenal failure
- Ovarian failure (minor ↓)



angiotensin converting enzyme (ACE) 3d 1.5mL

See aldosterone, Ca, comments, CRP, PO_4, PTH, renin, & vit. D.

Twice a week

8 – 52 U/L
Roche Cobas: Trinity Biotech

Use • **Diagnosis & monitoring of sarcoidosis.**

Background

- Largely from **pulmonary vascular endothelium**, but many tissues express ACE eg. renal epithelium, Leydig cells & **granuloma epithelioid cells** (descendents of activated macrophages).
- A **dipeptidyl carboxypeptidase** (an enzyme which cuts off the two amino acids at the carboxyl end of a peptide).
- ACE converts the inactive decapeptide **angiotensin 1** (produced by the action of renin on angiotensinogen secreted by the liver) to the octapeptide **angiotensin II** – a potent vasoconstrictor & stimulus of **aldosterone** secretion.
- ACE also **inactivates bradykinin** (a vasodilator).
- Levels are ↑ in only 60 % of sarcoid cases at diagnosis.

Causes of ↑

- sarcoid
- leprosy
- pulmonary fibrosis
- rheumatoid dis.
- myeloma
- berylliosis
- silicosis
- hyperthyroidism
- connective tissue diseases
- TB
- acute & chronic bronchitis
- atypical mycobacterial infection
- primary biliary cirrhosis
- ALD
- asbestosis
- Gaucher's disease
- histoplasmosis

Causes of ↓

- ACEIs (effect lasts approx. 12h after stopping)
- starvation
- hypothyroidism

anion-gap 1d See profiles

See bicarbonate, blood gases, Cl, ethylene glycol, K, lactate, Na & salicylate.

Daily

7 – 16 mmol/L
Tietz

Use • Ix the **cause of metabolic acidosis** (MA), but in practice this is usually known.

Background

- Anion gap (AG) estimates the **unmeasured anions** contributing to electro-neutrality ie.

AG = (serum **Na** conc. + **K** conc.) – (serum **chloride** conc. + **bicarbonate** conc.)

- AG is mostly **protein** anions with a little **lactate**, **urate** & **phosphate**, in health.

Causes of normal AG metabolic acidosis

- Renal Tubular Acidosis
- acetazolamide
- uretero-sigmoidostomy
- small gut fistulae & drainage
- chronic diarrhoea

Loss of bicarbonate from the body & a ↓ serum level, is compensated by ↑ renal Cl conservation & ↑ serum Cl.

Causes of ↑ AG metabolic acidosis

• lactic acidosis	lactate
• DKA	hydroxybutyrate
• methanol OD	formate
• ethylene glycol OD	glycolate
• aspirin OD	salicylate
• CRF	PO_4, SO_4

These unmeasured anions replace bicarbonate consumed by buffering ie. serum bicarb. ↓ but Cl does not ↑, as above.

anti-Mullerian hormone (AMH) 2d 1.5mL

Sent

pmol/L (AMH gen II Beckman Coulter)

Glasgow Royal Infirmary

See FSH, inhibin B, LH & oestradiol.

- **Any day of menstrual cycle** *cf.* FSH

Use

- Predicting oocyte production after ovarian stimulation in fertility therapy: serum AMH ≤ 5.4 pmol/L = low response, ≥ 25.0 pmol/L = high response. (NICE CG156)
- Predicting risk of **ovarian hyperstimulation syndrome.**
- **PCOS** management. .. ..
- Estimation of **ovarian reserve** / fertility for family planning.
- Ix of **disorders of sexual development** (DSDs).

Background

- A 140 kDa **homodimeric glycoprotein** structurally related to inhibin, TGF-β & activin.
- **Gender specific physiology:**

MALE

- Secreted by testicular **Sertoli** cells, it inhibits development of the **Mullerian ducts** into fallopian tubes, uterus, cervix & upper vagina.
- In **persistent Mullerian duct syndrome** (PDMS), mutation of the AMH gene or its receptor cause these structures to be present in otherwise normal males.
- **High serum AMH before age 2y**, progressive fall until the start of puberty, then a sharp fall. *cf.* female
- AMH & inhibin B can be used as markers of Sertoli cells in the Ix of **disorders of sexual development** eg. distinguishing cryptorchidism from anorchidism.

FEMALE

- AMH is secreted by **granulosa cells** of developing follicles.
- It **inhibits recruitment** & **growth** of more follicles, especially in response to FSH stimulation.
- **Serum AMH is low until puberty** when it ↑ & **then declines slowly** with age & falling number of primordial follicles. *cf.* male. At **menopause**, AMH becomes undetectable.
- **Ovarian reserve** (& fertility) falls with age. Available hormonal indices to aid family-planning:

- **Day 3 FSH**: the low oestradiol on day 3 makes FSH secretion greater in response to any ↓ feedback inhibition from ↓ inhibin secretion by declining ovaries (*cf.* menopause changes).
- **AMH**: **unaffected** by **menstrual cycle, pregnancy** & **OC pill**, unlike FSH, but the **wide variation of results** impairs interpretation of single results in individuals. *See below.*

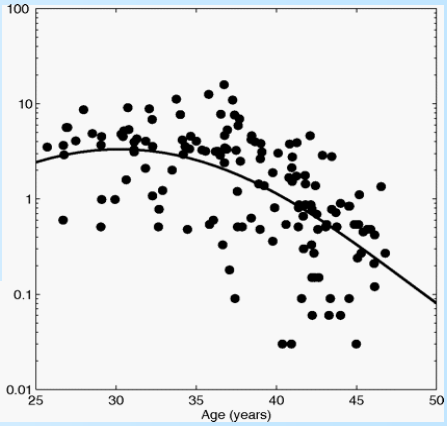
Causes of ↑

- **PCOS** (higher in anovulatory than ovulatory patients)
- ↑ levels are associated with ↑ risk of **ovarian hyperstimulation syndrome** (ascites, pleural effusion, hypovolaemia, DVT etc.).

Causes of ↓

- ↓ **ovarian reserve** ie. ↓ follicle abundance.
- **Aging**: number & quality of follicles ↓ with age

Age-related ↓ in AMH & **wide between-individual variation of results** at each age (note the log. scale) van Disseldorp 2008. However, within-individual variation is less ie. serial measurements can indicate the rate of decline of fertility & the age at menopause.



AST (aspartate aminotransferase) 3d See profiles

See albumin, ALP, ALT, ammonia, amino acids, AST/ALT ratio, bilirubin, GGT, cTnT, urea & vit. B6.

gold

Daily

Women	≤ 32 U/L
Men	≤ 40
Roche Cobas ASTL method sheet V10	

Use • Ix liver disease.

Background

- An **aminotransferase** (like ALT) which catalyses the exchange of an amino group from the amino acid **aspartic acid** to the keto acid **oxoglutarate**. AST catalyses the reverse reaction too.
- AST is a key player in **catabolism** & **anabolism of amino acids** from dietary & endogenous substrates.
- High AST levels exist in **hepatic mitochondria** & the **cytoplasm** of many other tissues eg. myocardium, brain, rbcs, skeletal muscle & kidneys
- ie. **AST is less specific than ALT** as a marker of liver disease.

Causes of ↑ (1 – 3x)

These are rough guidelines. Levels also depend on the scale of disease & the stage in the natural history.

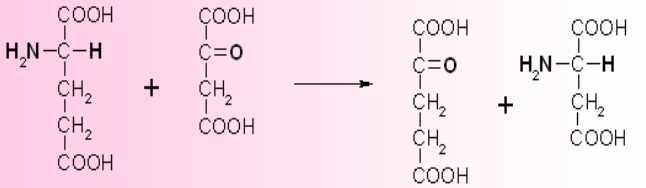
- *In vitro* haemolysis.
- Drug side effects eg. statins
- Lesser myocardial & skeletal muscle injury & disease.
- Physiologically in **neonates** (to approx. 1.5x)
- Some of the causes listed below:

AST/ALT ratio (De Ritis ratio).
Rough guide, best if levels are ↑↑

< 1	Normal & viral hepatitis
1 – 2	Cirrhosis
> 2	Alcoholic hepatitis

Causes of ↑↑ (2 – 10x)

- Skeletal muscle disease or injury
- Surgical muscle trauma
- Hepatic cirrhosis (AST may be normal)
- Glandular fever
- Cholestasis (AST may be normal)
- Fatty liver (steatohepatitis)
- Hepatic mets. (AST may be normal)
- Haemolytic anaemia



Causes of ↑↑↑ (10 – 100x)

- Acute viral & toxic hepatitis
- MI
- Circulatory collapse

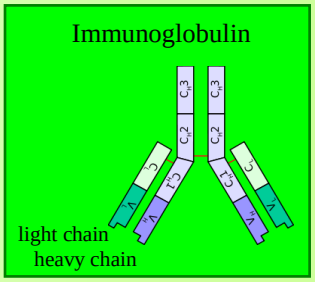
.

B

Bence Jones protein 3d 20mL Urine protein electrophoresis (UPE) Absent

See BJP quantitation, β_2M, cryoglobulins, free light chains, IgG subclasses, Igs, LDH & protein electrophoresis (serum) & (urine).

Every 1 – 2 d



- **BJP cannot be detected by protein dip-stix**.

Use • Ix myeloma

Background

- Immunoglobulin (antibody) molecules consist of 2 identical **light chains** of types **kappa** or **lambda** & 2 identical **heavy chains** (types **G, A, M, D** & **E**) linked by disulphide bonds.
- 1 heavy & 1 light chain together form an **antigen binding domain**.
- **B-cells synthesise antibodies** & chain synthesis is balanced.
- In **neoplastic** B-cells, light chains are often produced in excess.
- With a MW of **23kDa**, light chains readily enter the glomerular filtrate where they are termed **Bence Jones protein** & can be identified by electrophoresis of urine which has been concentrated in the lab. The separated proteins are identified by staining with antisera to specific chain types.
- Screen B-cell malignancy eg. myeloma with **serum *AND* urine** protein electrophoresis (BJP) because:

- **20%** of myelomas secrete **only light chains** ie. no paraprotein
- likelihood of **malignancy ↑ if BJP is present**, but it's not proof - *rarely* the cause can be benign.

- 1 – 5 % of myelomas are "**non-secretory**" ie. even BJP is absent. See β2 microglobulin.
- **MGUS** (Monoclonal Gammopathy of Unclear/certain Significance) does not show BJP, by definition, but **monitor BJP** 3 monthly if < 50y old or annually if elderly.
- In **BJ-myeloma**, use of serum paraprotein as a tumour marker is not an option & BJP quantitation has to be used despite its technical difficulty. See serum **free light chains**.

Causes of detectable urinary BJP

- **B-cell malignancy**: myeloma • lymphoma • plasmacytoma • macroglobulinaemia • CLL
- **Benign:** rare.

Bence Jones protein (quantitation) 3d 24h

Sent

Absent
Sheffield Protein Reference Unit

See BJP, free light chains & serum protein electrophoresis.

- **Not for screening**
- **Procedure**: Urinate in the toilet & note the time (can be any time). Collect all urine for the next 24h. The last one to collect is whatever can be passed at the noted-time next day.

Use

- **BJ-myeloma** tumour marker ie. for **monitoring** myeloma which secretes BJP but lacks a serum paraprotein.
- **DO NOT quantitate BJP** if there is a paraprotein to use as a marker of tumour bulk & response to therapy.

Background

- BJP quantitation is technically problematic & ordinarily adds little to the use of serum paraprotein as a marker of myeloma tumour bulk & response to therapy.
- With a **MW of 23 kDa**, free light chains readily enter the glomerular filtrate, where they are termed BJP.

beta hydroxybutyrate (βOHB) 3d 1.5mL

Sent

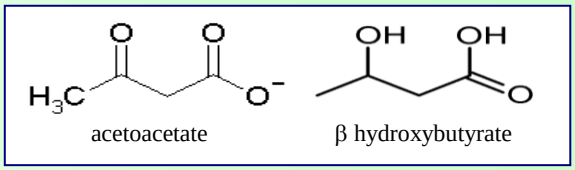
Refs & comment with result
Great Ormond Street Hospital

See anion-gap, carnitine, C-peptide, FFA, glucose, insulin & organic acids.

• **Take to lab. immediately** • Request **glucose, FFA & insulin** too.

Use

• **Ix of hypoglycaemia**. MUST be confirmed in fluoride plasma - ward glucose measurement is not good enough.
• Monitoring **ketogenic diets**.



Background & interpretation

• **free fatty acid** (FFA) released by **lipolysis** in starvation & insulin deficiency, supplies energy directly to many tissues eg. muscle, but **not the brain**.
• However, **ketone bodies** (β hydroxybutyrate & acetoacetate), synthesised from **acetates** from FFA catabolism in hepatic **mitochondria**, are taken up by the brain to provide 70% of its energy needs via the TCA cycle.
• **Acetoacetate** is truly a **ketone**: it has a ketone group (a carbonyl group, C=O, bonded to 2 carbon atoms). But **β-hydroxybutyrate**, which is formed by reduction of the carbonyl, strictly speaking, is not a ketone. However, they are both carboxylic acids & ↑ production in DKA is the main cause of the metabolic acidosis.
• **In hypoglycaemia** caused by:

↑ INSULIN ACTIVITY or insulin-like activity causes:

↓ plasma βOHB
↓ FFA

Because insulin { ↓ lipolysis (FFA release from adipose tissue). ↓ hepatic ketone body synthesis.

FATTY ACID OXIDATION DEFECTS eg. medium chain acyl-CoA dehydrogenase deficiency (**MCAD**) cause:

↓ insulin	(appropriate response to hypoglycaemia)
↑ FFA	(↓ insulin causes lipolysis)
↓ βOHB	(or disproportionately ↓ in view of ↑ FFA)

ie. ↓ **fat oxidation** & ↓ **ketone** body synthesis. If found, test urine organic acids & carnitines (Guthrie card).

IDIOPATHIC KETOTIC HYPOGLYCAEMIA causes:

↓ insulin	(appropriate response to hypoglycaemia)
↑ FFA	
↑ βOHB	(unlike fatty acid oxidation defects in which it is ↓)

beta 2 microglobulin (B2M) 3d 3mL

Sent

1.2 – 2.4 mg/L
Sheffield Protein Reference Unit

See BJP, free light chains & LDH.

• **For use by the Haematology Team only.**

Use

• Monitoring tumour bulk & response to treatment of **non-secretory myeloma** where the absence of serum paraprotein & Bence Jones protein limits the options for surveillance.

Background

• β2M is an **11kDa protein** component of the **HLA complex** on the surface of *all cells*.
• **Reabsorbed in the PCT** after readily entering the glomerular filtrate.
• Thus, ↑ urinary loss has been proposed as a marker of renal tubule dysfunction.
• In practice, β2M **degrades too readily** in urine at pH < 6.
• **Retention** of β2M in myeloma-associated **renal dysfunction** ↑ sensitivity of serum β2M as a tumour marker.

Causes of ↑

• ↓ GFR
• myeloma
• acute transplant rejection
• cancer (non-specific & variable link to tumour bulk)

Causes of ↓

• immunosuppressant Px • corticosteroids

bicarbonate 1d See profiles

See anion gap, blood gases, Ca ionised, chloride, comments & lactate.

Daily

22 – 29 mmol/L
Roche Cobas CO2-L method sheet V6

Use

• Ix of **hypokalaemia**.
• Calculation of **anion-gap**.
• Venous bicarbonate assay **usually adds little** to clinical data & results of other tests.

Background

• The measured bicarbonate (**total CO_2**) is actually a mixture of:
- **true bicarbonate** (HCO_3) - ▶
- **dissolved carbon dioxide** (CO_2)
- **carbonic acid** (H_2CO_3)
- **carbonate** (CO_3)
- **carbamino compounds**.

Bicarbonate on blood gas machines is calculated from H^+ & PCO_2 ie. the lab result is higher because it includes other forms.

$[H^+]$ is proportional to $\frac{[PCO_2]}{[HCO_3]}$

• Full acid/base evaluation requires **arterial blood gas** assays because without a P_{CO2} result, the effect of bicarbonate on pH or H^+ conc. is unknowable since it is determined by the *ratio* of bicarbonate to P_{CO2}.
• **Hypokalaemia** commonly occurs with **metabolic alkalosis** (bic. ↑) eg. hyperaldosteronism (1y & 2ndy).
• **Rarely**, hypokalaemia is associated with **metabolic acidosis** due to urinary loss of bic. & K ie. serum bic. is ↓ rather than ↑ with hypokalaemia eg. in renal tubular acidosis (**RTA**).

bile acids (bile salts) 3d 1.5mL

Daily

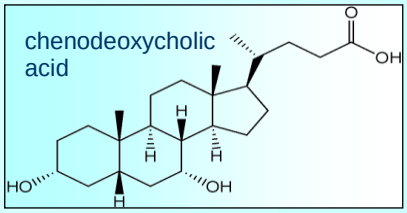
≤ 10 umol/L
Inverness Medical UK

See ALT, AST, bilirubin & urine protein.

- **Fasting needed** (levels ↑ after meals).

Use

- Ix & monitoring **cholestasis of pregnancy**.



Background

- **Cholic acid & chenodeoxycholic acid** (primary bile acids) are **conjugated** with the amino acids **glycine** or **taurine** before secretion into the gut where their **detergent** properties are necessary for micelle formation & fat-soluble vitamin absorption.
- Bacteria convert 1y to 2ndy bile salts ie. **deoxycholate** & **lithocholate**, respectively.
- All bile salts undergo **enterohepatic circulation** via the terminal ileum, 2ndy salts less than 1y ones.
- In **cholestasis of pregnancy**, bile salt levels have prognostic value for **fetal survival** & contribute to the decision to deliver early in order to ↓ fetal risk.

Cause of ↑

- Hepatobiliary disease, especially cholestasis & notably cholestasis of pregnancy.
- Feeding.

bilirubin (direct) 3d See profiles
See ALP, ALT, AST, bilirubin (total), GGT, **MRI** & urobilinogen.

Daily

≤ 5.0 umol/L or <15 % of total bili.
Roche Cobas BILD2 method sheet: V4

Background

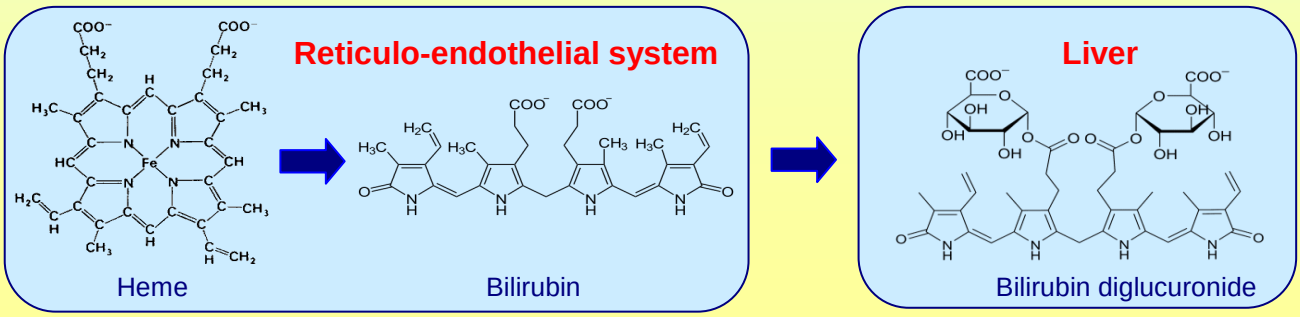
- Bilirubin (from catabolism of haem, see total bilirubin) is **lipophilic** & carried in the circulation to the liver bound to plasma proteins eg. albumin & hemopexin.
- In the **liver**, **water solubility is ↑ by conjugation**, especially with **glucuronic acid**, before biliary excretion. Obstruction of this (**cholestasis**) causes ↑ amounts of conjugated bilirubin to re-enter the general circulation.
- Conjugated bilirubin in plasma is **less protein-bound** than unconjugated bilirubin, thus **enters urine** where it is the bilirubin form detected by test strips.
- **Direct** bilirubin assays measure it under conditions in which the **water-soluble conjugated forms react directly** with reagents without the "accelerators" necessary for unconjugated bilirubin to react.
- Assay specifity is < 100% ie. **direct** & **conjugated bilirubin are not synonymous**, but difference is minor.

Causes of ↑

- Intra & extrahepatic **cholestasis** eg.

- gall stones
- hepatitis
- drug effects
- space occupying lesions
- Dubin-Johnson & Rotor syndromes.

NB. In intrahepatic cholestasis, the bilirubin load may be cleared without ↑ in serum bilirubin if there are sufficient unobstructed biliary canaliculi ie. the biochemical signs may be limited to ↑ALT, ↑ALP & ↑GGT.



bilirubin (total) 3d See profiles

See albumin, ALP, ALT, AST, bili. (direct), carotene, comments, GGT, haptoglobin, hemopexin, **MRI**, phenobarb., porph., urobilinogen, vit. A & xanthochromia.

Daily

Adults	≤ 21 umol/L
Children > 1m	≤ 17
Roche Cobas BILT2 method sheet V5	

Uses

- Serum total bilirubin results plus other LFTs & clinical & imaging data, are sufficient LFTs for most cases.
- Assay of **direct bilirubin** (conjugated) may aid diagnosis of the cause of jaundice, especially in paediatrics.

	Prem. **neonates**	Term **neonates**
24h	17.1 – 102.6 umol/L	34.2 - 102.6 umol/L
48h	102.6 - 136.8	102.6 - 119.7
3 - 5d	171.0 - 256.5	68.4 - 205.2
Roche: Reference Ranges for Adults and Children 04		

Background

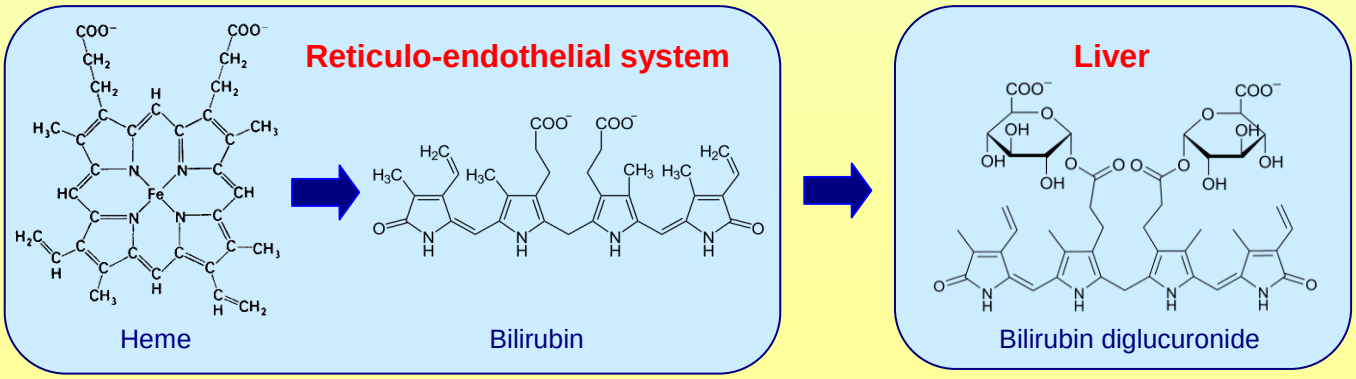
- Serum total bilirubin is the **sum of all forms** ie. conjugated + unconjugated.
- Bilirubin is the **remnant of haem** after removal of the ferrous iron & release of haem from proteins eg. haemoglobin, myoglobin, cytochromes, catalase.
- This takes place in the **reticuloendothelial system**, especially the spleen, where each tetrameric Hb molecule degrades to release 4 haems (Hb figure)
- These form 4 unconjugated bilirubin molecules, which **bind to albumin** for transport to the liver where they enter hepatocytes by an active process.
- In hepatocytes, **lipophilic** bilirubin is **made water soluble by conjugation** with glucuronic acid to form mono & diglucuronides before they are excreted in bile.
- Use of "accelerators" in the assay enable all forms of bilirubin to be measured, not just water soluble ones.

Causes of ↑

Consider in terms of unconjugated & conjugated (≈ direct bili) components of total bilirubin**:**

Type of hyperbilirubinaemia	Causes
Unconjugated. *Not assayed, but approx. = total – direct bili. conc.*	↑ bilirubin production eg. haemolysis
	↓ hepatic uptake eg. hepatitis
	↓ glucuronidation eg. Gilbert's & Crigler-Najjar syndromes.
Conjugated *direct bilirubin*	↓ excretion due to obstruction eg. drug effect, oedema, stones

In reality, hyperbilirubinaemia is mixed & the degree may vary with the natural history of the illness eg. in hepatitis the initial unconjugated bilirubinaemia due to impaired hepatocyte function is followed by ↑ levels of conjugated bilirubin resulting (largely) from oedema & a cholestatic element.



biotinidase 0d 1.5mL

Sent

See lactate (CSF).

2.5 – 10.5 U/L
Sheffield Children's Hospital

Use Ix babies & children for biotin responsive epilepsy.

Background

- **Biotin** is a cofactor needed for the function of 4 key **carboxylases** in:
  - fatty acid synthesis
  - amino acid catabolism
  - gluconeogenesis eg. *pyruvate carboxylase*.
- It binds covalently to the carboxylase & subsequently must be released by the enzyme ***biotinidase*** for reuse, otherwise deficiency develops, which causes fits, developmental delay, ataxia, SIDS etc.
- *Biotinidase* is **ubiquitous**, including free in plasma.

Biotinidase deficiency

- 1:60k births, but **treatment with biotin** is effective.
- Variants which cause **partial loss** of biotinidase function give **milder** phenotypes which present **later** & may require stress to precipitate them eg. **fever or fasting**.
- A **severe neonatal form** of apparent biotinidase deficiency can also be caused by loss of function of *holocarboxylase synthetase*, the enzyme which adds biotin to the carboxylase.

blood gases (arterial) 0d Heparinised blood gas syringe

Daily

See anion gap, carboxyhaemoglobin, bicarbonate, Ca ionised & metHb.

pH	7.35 - 7.45
PCO_2	4.3 - 6.4 kPa
PO_2	9.3 - 14.4 kPa
BE	–2.5 to +3.0 mmol/L
IL Gem 4000 reference guide + Tietz	

- **No bubbles**
- **Remove needle** & apply a cap
- **Do not send in the "air tube"**
- **TRAINING IS ESSENTIAL** – contact Chem. Path. ext 1691.
- PCO_2, PO_2, pH, bicarbonate & carboxyHb are available on **machines in specialist units**

- **The lab. measures blood gases too:**
  - a request form & a capped & labelled syringe are needed.
  - take to the lab. by hand – **don't use the air-tube**.
- Only PCO_2, PO_2 & pH are actually measured. Bicarbonate is calculated from them & it will differ from the lab. result found by direct assay in venous (not arterial) blood.

BNP (B type natriuretic peptide) 3d 1.5mL

Twice a week in-house

Actually N-terminal proBNP. See **MRI** & cTnT.

For **exclusion** of cardiac disease.

	45 - 59 y	> 59 y
women	≤ 164 ng/L	≤ 225 ng/L
men	≤ 100	≤ 172
97.5th centiles in asymptomatic, **echo. –ve**, healthy people. These are also the thresholds for detection of LV dysfunction. *Galasko G. et al 2005*.		

Suggested by **NICE CG108** for Ix **heart failure**, but does not take account of age, gender & asymptomatic disease. The higher ref. values give ↓ sensitivity & ↑ false negs ie. ↓ **power for excluding cardiac disease**.

Normal	< 400 ng/L
Raised	400 – 2000
High	> 2000
NICE CG108 No references iven ***?echo used***	

More normative data showing the importance of **gender** & **age**, but usage is weakened by failure to exclude **asymptomatic disease**.

	AGE (y) (*97.5th centiles*)				
	18 – 44	**45 – 54**	**55 – 64**	**65 – 74**	**≥ 75**
women	≤ 130	≤ 249	≤ 287	≤ 301	≤ 738 ng/L
men	≤ 85.8	≤ 121	≤ 210	≤ 376	≤ 486 ng/L
Roche Cobas proBNPII method sheet V8: 2264 asymptomatic normals, ***no echo.***					

Children

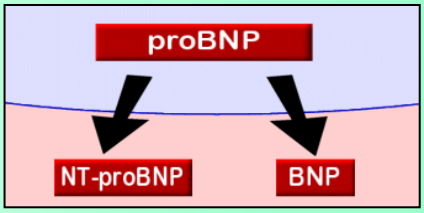
Age (y)	**1 - 3**	**4 - 6**	**7 - 9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
NT proBNP ng/L	320	190	145	112	317	186	370	363	217	206	135	115
97.5th centiles. Roche Cobas proBNPII method sheet V8, Albers S et al 2006.												

Importance of reference population

- NT-proBNP **levels ↑ with age,** at least partly due to ↑ prevalence of asymptomatic cardiac dysfunction.
- Thus, references are **higher if echocardiography is not used** to exclude asymptomatic cases.
- Serum levels are **higher in women than men**.

Use

- Ix of **heart failure/LV wall stretch**.



Background

- BNP is secreted by the heart in response to **ventricular wall stretch**.
- Stimulates **urinary sodium excretion**.
- A large & **inactive precursor** protein (proBNP) is cleaved before secretion, to release **equimolar** amounts of **BNP** & an inactive amino-terminal fragment, **NT-proBNP**.
- NT-proBNP is equivalent to BNP, but **less vulnerable to degradation** & the sample requirements are simpler.
- Serum NT-proBNP ↑ in many heart diseases, **not just heart failure**.
- The high sensitivity & specificity of NT-proBNP assay gives results with a **high –ve predictive value** (96 99%) ie. assay is **good for "ruling out" cardiac disease** & reducing use of echocardiography.
- The +ve predictive value (PPV) is 62% ie. NT-proBNP is **not so good for "ruling in"** cardiac disease. Positive results need confirmation eg. echocardiography.
- The value of other uses is less clear eg. prognostication in CCF & ACS, & monitoring the response to therapy.

Causes of ↑

- LV dysfunction
- ACS
- valvular-disease
- arterial hypertension
- female (related to oestrogen?)
- sepsis.
- LVF
- MI
- cardiomyopathy
- ↑ Na intake
- < 1h after exercise
- CCF
- PE
- bradycardia
- age
- ↓ GFR

C

CA 12.5 (cancer antigen) 3d 1.5mL See profiles

Daily

See AFP, CA153, CA199, CEA, hCG, **MRI**, PLAP & SCC Ag.

- **DO NOT use other ref. ranges.**
- **Results depend on the particular antisera used in the assay.**

≤ 35 kU/L (95th centile)
Roche Cobas CA 125 II method sheet V16

Use

- Monitoring **ovarian tumour** bulk & response to therapy after diagnosis by other means.
- **Not for diagnosis** or for **screening** low risk patients.

Background

- CA12.5 is a **glycoprotein** expressed in the mucus of tissues of coelomic epithelial origin:
  - ovary
  - fallopian tubes
  - endometrium
  - endocervix
  - peritoneum
  - pleura.
- For **monitoring** diagnosed ovarian carcinoma and women at high risk of it.
- plasma **half life = 9 d**
- **Not for diagnostic use:** inadequate sensitivity (too many false –ves) & specificity (too many false +ves) .

Causes of ↑

- ***any* peritoneal or pleural disease** eg. infection, inflammation, infiltration, ascites, pleural effusion….
- endometriosis
- pregnancy
- colonic diverticulitis (can be >500 kU/L)
- benign ovarian disease eg. cyst
- melanoma
- renal failure
- LVF (100-150 kU/L)
- hepatic cirrhosis
- carcinoma of breast, endometrium, g.i.t., ovary
- early menstrual cycle
- CCF (200-300 kU/L)
- hepatitis

CA 15.3 3d 1.5mL See profiles

Daily

≤ 25 kU/L (95th centile)
Roche Cobas CA 15-3 II method sheet V17

See AFP, CA125, CA199, CEA, hCG, **MRI** & SCC Ag.

Do not use other ref. ranges. Results depend on the particular antisera used in the assay.

Use

- Monitoring of **breast carcinoma** bulk & response to therapy after diagnosis by other means.
- **Not for diagnosis** or for **screening** low risk patients.

Background

- Highest levels of this epithelial **mucoprotein** are seen in **breast carcinoma**.
- ↑ is also seen in other malignant & **benign** diseases ie. it has **low specificity** for breast tissue.
- After diagnosis by other means, use CA15.3 **for monitoring** recurrence, tumour bulk & therapeutic response.

Benign causes of ↑

- benign disease of breast, liver, pancreas & gall bladder
- pregnancy
- CRF
- TB
- benign gynae. & urological diseases.

Malignant causes of ↑ Carcinoma of:

- breast
- liver
- ovary
- uterus
- pancreas
- prostate
- lung
- gastrointestinal tract

CA 19.9 3d 1.5mL See profiles

Daily

≤ 34 kU/L (97.5th centile) or ≤ 27 kU/L (95th centile)
Roche Cobas CA 19-9 method sheet V21

See AFP, CA125, CA153, CEA, hCG, **MRI** & SCC Ag.

Do not use other ref. ranges. Results depend on the particular antisera used in the assay.

Use

- **Monitoring pancreatic** & colorectal **carcinoma** bulk & response to therapy.
- **Not for diagnosis or screening** low risk patients (insufficiently sensitive or specific).

Background

- CA19.9 is a **mucoprotein** normally expressed in the epithelium of the pancreas, stomach, liver, biliary tract, bronchial tree & salivary glands. There are low levels in the colon, rectum & female genital tract too.
- It can ↑ in **pancreatic, hepatobiliary** & **gut cancer**.
- 5 – 10 % of the population **do not express CA19.9**.
- **Sensitivity** & **specificity** can be improved by measuring **CEA** too.

Causes of ↑

- Highest levels occur with **pancreatic carcinoma**, but **gall stone cholestasis** can produce similar extreme levels, although they fall to normal within 5 w of relief of obstruction.
- Smaller ↑ can be seen in:

- hepatitis
- cholecystitis
- hepatic cirrhosis
- chronic pancreatitis
- carcinoma of the organs mentioned above in “Background”.

caeruloplasmin 3d 3mL
See acute phase response, copper (plasma) & copper (urine).

Sent

< 4m	0.09 – 0.56 g/L
4m – 1y	0.14 – 0.41
1y – 10y	0.24 – 0.47
10y – 13y	0.18 – 0.27
> 13y	0.20 – 0.60
Adult	0.20 – 0.60
Sheffield PRU (5th-95th cent)	

Use
- Ix of **Wilson's disease**.

Background
- **6 atoms of copper** (Cu) bind to each caeruloplasmin protein molecule.
- **95%** of circulating copper is carried by caeruloplasmin.
- **Defence from oxidative injury** during inflammation is probably its role rather than **Cu transport**.
- Synthesised by **hepatocytes** (the major source of plasma caeruloplasmin), monocytes & glial cells.
- The **acute phase response** ↑ levels 2 – 3 fold, potentially **masking the ↓ levels of Wilson's disease**.

Causes of ↓
- Cu deficiency • Wilson's dis. • impaired hepatic function • protein losing & catabolic states.

Causes of ↑
- Acute phase response • oestrogens eg. gender, OCP, pregnancy, HRT.

Wilson's disease
- In this, the **Cu transporter** ATP7B is defective, leading to:
- ↓ biliary excretion of Cu (the main excretion route)
- ↓ incorporation of Cu into caeruloplasmin
- ↓ plasma copper concentration
- ↑ liver Cu content
- ↓ secretion of caeruloplasmin into the circulation, but Cu exits the liver by non-specific routes
- ↑ accumulation of Cu in tissues eg. brain, eye & kidney.
- ↑ urine Cu excretion
- Presentation:
- 45% **hepatitic** eg. asymptomatic ↑ ALT, acute & chronic hepatitis, cirrhosis & fulminant hepatic failure. Most cases present at 3 – 18y of age (**v. rare after age 50y**).
- 45% **neurological** eg. dysarthria, tremor, gait disturbance & rigidity. Presents later (**rare <10y old**).
- 10% **psychiatric**.
- Inheritance: **autosomal recessive**. Most patients are **compound heterozygous**.
- **DNA analysis** is best used to test relatives of index cases. P**henotype-genotype correlation is not good enough** for DNA analysis to be the primary diagnostic tool.
- Roughly, mis-sense mutations cause more progressive & later onset disease, than non-sense variants.
- Very low plasma Cu & caeruloplasmin *usually* adequately confirm the diagnosis.
- Particularly **in hepatic cases**, serum **caeruloplasmin may be in the ref. range** because immunoreactive but non copper carrying caeruloplasmin precursor (**apocaeruloplasmin**) may be secreted. 24h **urine copper** assay can clarify things, since it is usually ↑ in Wilson's dis. NB. urinary Cu excretion can ↑ in **cholestasis of any cause** & it can be normal in Wilson's disease.

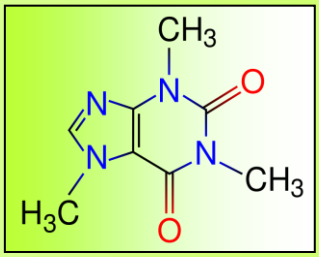
caffeine 3d 1.5mL

Sent

5 – 20 mg/L
St Helier Hospital

See therapeutic drug monitoring & theophylline.

- **Pre-dose sample.**



Use

- Optimisation of caffeine therapy

Background

- Caffeine is a **CNS stimulant** used to treat **apnoea** of prematurity.
- It is related to **theophylline**, but is less toxic & more predictable to use, despite wide variation in its elimination **half-life (40 – 230h)**.
- 85% is excreted unchanged in **urine**.
- The proportion metabolised by **hepatic** oxidation & demethylation, rises with liver maturation after 3m of age.

calcitonin 0d 4mL

No gel

Sent

< 2 mIU/L
Sheffield PRU

See Ca, PTH, thyroglobulin, TSH & vit D.

- **Keep on ice.**
- **Take to lab. immediately.**
- Sample tube **MUST NOT contain gel** ie. red cap without a yellow ring.

Use

- Monitoring **medullary thyroid carcinoma** & its **response to therapy.**

Background

- A 32aa peptide of unclear function in man, secreted by thyroid **parafollicular C cells**.
- Formerly used for screening relatives of known cases eg. **MEN II**
- **DNA analysis** of the **RET-proto-oncogene** is used now. **Please give details of the index case**.

Causes of ↑

- medullary carcinoma of thyroid
- pregnancy
- renal failure
- pernicious anaemia
- SCC of lung
- breast carcinoma
- renal tubular carcinoma
- carcinoid tumours

calcium (serum, adjusted) 3d See profiles
See albumin, ALP, Ca (total), Ca (ionised), Ca (24h urine), comments, PO_4, PTH & vit. D.

Daily

Use
- An **aid to interpretion of total Ca conc**. when albumin conc. is abnormal.

Background
- **Approx. half** of the total serum calcium is bound to albumin.
- Variation of the **albumin conc. thus affects the total Ca level**, which can cause diagnostic difficulty.
- Adjusted Ca is an **estimate** of what the total Ca would be **if the albumin was restored** to "normal" ie. 40g/L.
- It is not intended to be more than **an aid to interpretation** of serum total Ca, particularly when factors with opposing influences co-exist eg. wasting & neoplastic hypercalcaemia.

Calcium (ionised) Heparinised blood gas sample.
See bicarbonate, blood gases, Ca total, Ca adj.

1.15 – 1.27 mmol/L (arterial & venous blood)
IL GEM 4000 documents

- **Available on blood gas machines, NOT THE LAB.**

Background
- Plasma calcium is present as **Ca^{++} ions**.
- Approx. half binds to negatively charged (anionic) sites on albumin.
- The remainder (called ionised or free calcium) remains in solution & is measured by an **ion selective electrode**
- ie. **ionised Ca is approx. HALF the total Ca** measured by the lab.
- H^+ ions compete with Ca^{++} ions to bind to albumin's anionic sites.
- Alkalaemia thus ↑ Ca binding & ↓ free ionised Ca.
- Ionised Ca is the bioactive fraction & a ↓ can cause symptoms eg. tetany, +ve Chvostek's sign.

Causes of ↑
- Causes of ↑ total Ca adjusted for albumin conc.

Causes of ↓
- Causes of ↓ total Ca adjusted for albumin conc. • alkalaemia • citrate

calcium (serum, total) 3d See profiles

Daily

See ACE, Al, albumin, ALP, amylase, Ca (adj), Ca (ionised), calcitonin, Ca (24h U), comments, digoxin, Li, Mg, **MRI**, PO_4, PTH, stones & vit D.

0 - 10d	1.90 - 2.60 mmol/L
10d - 2y	2.25 - 2.75
2y - 12y	2.20 - 2.70
12y - 18y	2.10 - 2.55
18y - 60y	2.15 - 2.50
60y - 90y	2.20 - 2.55
> 90y	2.05 - 2.40
Roche Cobas CA2 method sheet V2	

Background

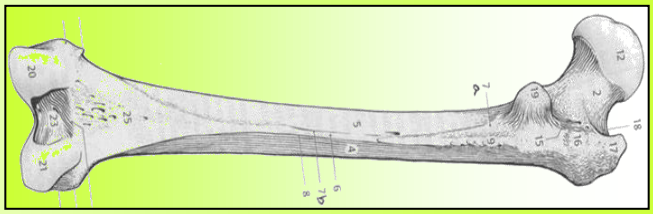
- Approx. half of serum total Ca is protein bound & half is free (ionised).
- **Ionised Ca** is biologically active & regulated by –ve feedback, PTH, vit. D, kidneys, bone, diet & the small intestine.
- Ca bound to albumin is biologically inactive. Thus ↑ albumin eg. due to prolonged tourniquet usage, will ↑ total Ca without physiological effect. ↓ albumin does the opposite.
- **Adjusted calcium** is an estimate of what the total Ca would be if the albumin was "normal" ie. 40g/L.

Causes of ↑

- ↑ albumin
- primary hyperparathyroidism
- tertiary hyperparathyroidism
- hypervitaminosis D
- sarcoidosis
- thiazides
- lithium
- neoplastic invasion of bone
- humoural hypercalcaemia of malignancy (PTH-RP)
- hypocalciuric hypercalcaemia
- immobilisation with hypermetabolic bones eg. Paget's & thyrotoxicosis
- milk-alkali syndrome

Causes of ↓

- ↓ albumin
- vit. D deficiency
- CKD
- hypoparathyroidism (post-surgical is commonest)
- ↓ Mg



calcium (24h urine) 3d acidified 24h urine

Daily

2.5 – 7.5 mmol/24h
Roche Cobas CA2 method sheet V2

See Ca adj., Ca (total), Ca (U conc), comments, PO_4 (24h U), PTH, stone analysis & UA (urine).

- **Plain bottle.** Acid is added in the lab. on its return.
- **Procedure**: Urinate in the toilet & note the time (can be any time). Collect all urine for the next 24h. The last one to collect is whatever can be passed at the noted-time next day.

Use

- **Poor correlation with nephrolithiasis**.
- Serum Ca & PO4 (& PTH as necessary) are more useful.
- Ix of **hypocalciuric hypercalcaemia** (24h Ca excretion is typically ↓, not ↑, as in primary hyperparathyroidism)
- Daily calcium losses span a wide range of values & are related to Ca & protein intake & phosphate excretion.

calcium (urine conc.) 3d acidified 20mL **Children only** Daily

Ca (24h urine) & PTH.

- **24h urine Ca assay is superior.**

Calcium concn.	1.7 – 5.3 mmol/L
Ca/creatinine ratio	< 0.40 mmol Ca/mmol creat.
	> 0.57 *suggests* hypercalciuria
Tietz	

Use

- Ix of **familial hypocalciuric hypercalcaemia** (FHH)

Background

- The uncertain **dilution** of urine degrades the use of Ca concentration as an index of Ca excretion.
- Expression of Ca excretion as a **Ca/Cr ratio** reduces dilutional effects.
- 24h Ca excretion is the best measure, but a 24h urine sample is hard to collect reliably eg. children.
- **FHH** is suggested by**:**

- hypercalcaemia
- family history
- Ca/Cr ratio < 0.01
- PTH in the upper half of the ref. range or even a little raised.

- FHH can resemble primary hyperparathyroidism, but urinary Ca excretion is low, not high as in the latter.

calprotectin 3d a few grams of fresh faeces Sent

See elastase & **MRI**.

- **MUST have its own sample.**
- Clinical details needed.

0 – 50 ug/g of faeces
Royal Sussex University Hospital

Use

- **Distinguishing IBS** (irritable bowel syndrome) **from IBD** (inflammatory bowel disease) NICE DG11
- Early detection of **IBD relapse**
- Selection of children for **endoscopy**.
- **Screening children for organic causes** of gut symptoms eg. constipation.

Background

- **Neutrophil** granules contain abundant calprotectin, a **calcium binding protein**, which resists g.i. degradation
- At sites of **bowel inflammation,** degranulation releases it into the gut .

Causes of ↑

The clinical context is important for interpretation.

- IBD
- g.i. infection / inflammation
- polyps
- neoplasia
- NSAIDs

carbamazepine 3d 1.5mL

See drugs & TFTs, lamotrigine, MHD, phenobarb., sirolimus, TDM, valproate & zonisamide.

- **Pre-dose sample.**

Daily

4.0 – 10.0 mg/L
Roche Cobas CARB2 method sheet V7

Use

- Optimisation of **therapy** & **freedom from side effects**, if this cannot be done clinically.
- Levels which achieve this vary considerably between patients & with co-prescription eg. in adults, **combination therapy**: 4 – 8 mg/L ……. **mono-therapy**: 8 – 12 mg/L.

Background

- Carbamazepine stabilizes voltage-gated Na channels in the inactivated state, ie. neurones become less excitable.
- For treatment of **epilepsy**, trigeminal **neuralgia** & **bipolar** affective disorder.
- 70% protein bound
- Plasma **half-life = 8 – 60** h.
- Metabolised by hepatic ***cytochrome P450*** to **carbamazepine epoxide** (CE) (**bioactive**, 5–8 h half-life) which is inactivated by microsomal ***epoxide hydrolase (EH)***. Carbamazepine induces its own metabolism & levels should be tested 2 - 3 months after start of Px.
- These enzymes underlie important **drug interactions:**

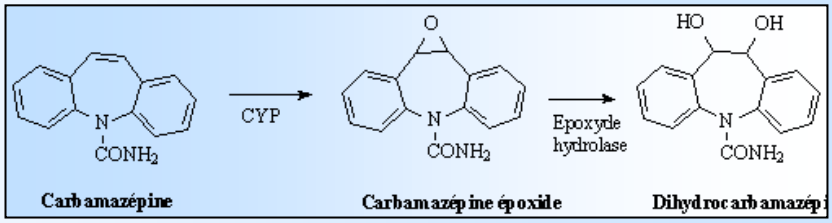
- Carbamazepine clearance ↑ & levels ↓ after **induction** of degradative enzymes by phenobarbitone, phenytoin & carbamazepine itself.
- Similarly, enzyme induction by carbamazepine causes ↓ phenytoin, valproate & zonisamide levels.
- Valproate & lamotrigine inhibit EH causing ↑ carbamazepine epoxide levels & ↑ clinical effects.

Carbamazepine half-life (hrs)	
normal	24 - 34
with valproate	59 - 70
with phenytoin	13 - 14
with carbamazepine	13 - 14
CRF	40 - 60
hepatic impairment	26 - 148

Biochemical side effects

- ↓ serum Na
- SIADH
- cholestasis
- hepatitis
- renal dysfunction.

.



carbohydrate deficient transferrin 3d 2mL

(CDT) See CK, ethanol, GGT, iron satn & tau protein.

Sent

≤ 2.6 % of total transferrin
Sheffield Protein Reference Unit

Use

- **Ix of chronic alcohol abuse**

Background

- Transferrin is a **glycoprotein** (β1 transferrin).
- Chronic **ethanol excess inhibits glycosylation**, especially with glucosamine.
- This ↑ the fraction of total transferrin which is CDT.
- **A marker of chronic ethanol abuse**, CDT is specific (98%) & sensitive (90%).
- CDT is used by agencies eg. **DVLA** & occupational health depts., for **monitoring ethanol abstinence**.
- Transferrin in **CSF** is naturally less glycosylated than in plasma & is known as **tau protein** or β2 transferrin. This is used to identify watery fluid leaking from the nose or ear, as CSF.

Causes of ↑

- chronic ethanol excess
- congenital transferrin variants
- congenital glycosylation deficiency (an inborn error of metabolism)
- oestrogen eg. HRT.
- chronic liver disease
- ↓↓ ferritin

carboxhaemoglobin 0d Heparinised blood gas syringe Daily

See blood gases.

- Can be venous **EDTA blood too.**

Nonsmokers	≤ 3 %
Smokers	≤ 10
Tietz	

Use • Ix of carbon monoxide (CO) poisoning.

Background

- The affinity of **carbon monoxide** for the ferrous ion in the haem of haemoglobin is 218x that of oxygen.
- Breathing only 0.1% CO in air, converts 50% of Hb to carboxyhemoglobin which **cannot transport oxygen**.
- Breathing air (21% oxygen) eliminates half the CO in blood in about 3h. Quicker with ↑ P_{O2}.
- Carboxyhaemoglobin is measured by **co-oximetry** (spectroscopy) on a blood gas machine.

Rough clinical correlations if health & Hb are otherwise normal	
20 % carboxyHb of total Hb	headache, lassitude
20 - 50	progressively severe headache & fatigue
50	muscle weakness, vertigo, SOB++, fainting
50 - 70	unconsciousness, death will follow
80	death imminent

Causes of ↑

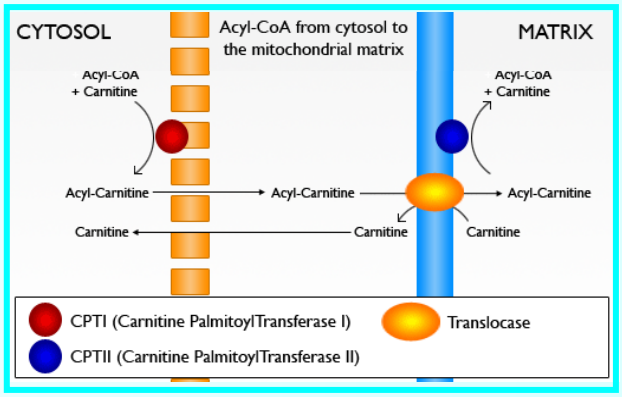
- smoking
- blood in gut
- CO poisoning
- exercise
- haemolysis
- ↓ energy intake.

carnitine (total, free & acyl) 0d Guthrie card Sent

Refs & comment sent with result
Great Ormond Street Hospital

See amino acids, ammonia, β-hydroxybutyrate, C-peptide, FFA, glucose, insulin & organic acids.

- Measure plasma **glucose too**.
- **Fast** for 10 – 12h.
- Sample **during a hypoglycaemic episode**, if poss.



Use

- Ix hypoglycaemia in babies & children

Background

- **Fatty acids** form esters with **coenzyme A** in the outer mitochondrial membrane.
- The resulting acyl-CoA reacts with **carnitine** to form **acyl-carnitine**, which then crosses to the inner membrane where the carnitine is **recycled** & acyl-CoA is reformed & enters the mitochondrion where the fatty acid is oxidised.
- Carnitine metabolism is crucial to **fatty acid oxidation** & ketone body production.
- Rare **defects** of carnitine metabolism can cause **impaired glucose-sparing** & **hypoglycaemia** when there is fasting, vomiting or intercurrent illness. Ketone body formation is ↓ & plasma fatty acid levels are relatively ↑. Assays of plasma **free** & **acylated** forms of carnitine help to locate the defective step in their metabolism.

carotene (beta) 0d 1.5mL

Sent

0.19 – 0.89 umol/L
City Hospital Birmingham

See bilirubin & vitamin A.

- **Protect from light**

Use

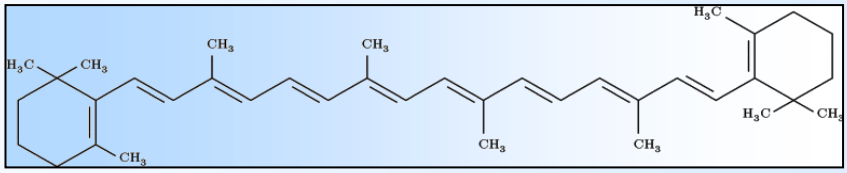
- Ix of patients (children especially) with yellow skin, but normal serum bilirubin levels eg. suspected **familial carotinaemia** or **excessive intake** of carotene rich food.

Background

- Many coloured plants contain carotene, but **carrots** are the most readily available & abundant source.
- Carotene is a key **precursor for vitamin A synthesis**.
- Ordinarily, high carotene intake does not cause vitamin A toxicity because the synthesis is inhibited by the vit. A produced. Excess carotene is excreted.
- However, there are case-reports of **hypervitaminosis A** with pathologically gross over-consumption of carrot juice because of its high level of carotene & the ease of drinking large volumes.

Causes of ↑

- Hypothyroidism
- CKD
- Familial carotinaemia
- Over zealous intake



Causes of ↓

- Malabsorption
- Malnutrition

CART 0d 6mL

Sent

< 85 pmol/L
Charing Cross Hospital

See gut hormone profile.

- **Does not need ice or aprotinin.** Bech P et al 2008

catecholamines 3d acidified 24h urine

Sent

See VMA.

- **Plain bottle.** Acid is added on its return to the lab.

CEA (carcinoembryonic antigen) 3d 1.5 mL See profiles

Daily

See AFP, CA125, CA153, CA199, hCG, **MRI** & SCC Ag.

- **Do not use other reference ranges.**
- **Method-specific results.**

	20 – 69 y old	**≥ 40 y old**
Non-smokers	3.8 ug/L	5.0 ug/L
Smokers (current)	5.5	6.5
Roche Cobas CEA method sheet V6		(95th centiles)

Use

- Monitoring recurrence & response of **colorectal cancer** to therapy.
- **NOT for diagnosis or screening**.

Background

- A 180 kDa **glycoprotein** on the surface of gastro-intestinal **epithelial cells**, especially in the **colon & rectum**.
- **Expressed ++ in fetal life** (like AFP), but much less *ex utero*.
- **Dysregulation in neoplasia** can cause expression to return.
- Beware **false + ves** from benign disease & assay interference (as with all tumour markers).
- **False – ves** too ie. **a normal CEA result does not exclude even gross disease**:

- poorly differentiated carcinomas may not secrete CEA.
- 28% of patients with non-resectable colorectal disease have normal serum CEA.

- CEA has **poor specificity** & **sensitivity** for early cancer detectionn ie. **do not screen low risk populations** for asymptomatic disease.
- **DO NOT delay referral** of patients with symptoms of lower g.i. cancer, by tests other than FBC (NICE CG 27).
- CEA's value lies in **monitoring** remission & the response to therapy, **not diagnosis.**
- **Non-colorectal cancers** which do not, or cease to express specific markers, can be **monitored too** eg. carcinoma of **bronchus & breast**, because of the low specificity of CEA.
- Plasma **half-life = 3 – 5 d** ie. levels should return to normal in 3 – 4 w after complete excision.

Causes of ↑: malignant

- colon
- breast
- bronchus
- neck
- thyroid
- stomach
- bladder
- head
- some ovarian cancers.

Causes of ↑: non-malignant (rarely >10ug/L)

- pregnancy
- smoking
- cirrhosis
- ulcerative colitis
- emphysema
- Crohn's dis.
- pancreatitis
- gastric ulcer
- pneumonia
- chr. hepatitis
- ALD (alcoholic liver disease).

.

C1 esterase inhibitor (C1 INH) 0d 1.5mL

Sent

See acute phase response, C3, C4 & CRP.

C1 INH	**functional activity**	40 – 150 %
.. ..	**mass conc.**	0.15 - 0.35 g/L
complement	**C3**	0.75 - 1.65 g/L
.. ..	**C4**	0.14 - 0.54 g/L
Sheffield Protein Reference Unit		

Use

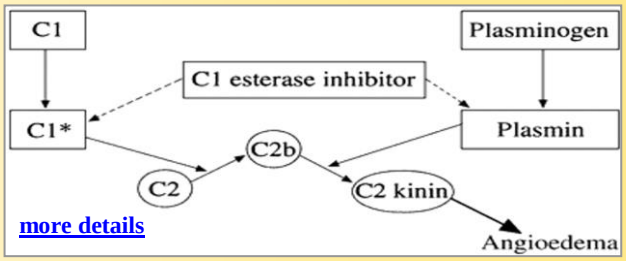
- Ix of **hereditary angioedema**

Background

- C1 INH is an **inhibitor of serine proteases** (serpin), which counters the esterase activity of the activated form of the first component of complement (C1) in order to **limit injury to normal tissue**. Not surprisingly, C1 INH levels ↑ with the acute phase response (like CRP).
- C1 INH **also inhibits**:

- factor XIIa,
- the generation of kallikrein
- kallikrein itself
- plasmin
- prothrombin activator

ie. it is a key regulator of the inflammatory response.



more details

- **Hereditary angioedema** (Osler's disease):

- 1:50k caucasians
- autosomal dominant
- painless non-pruritic swellings on limbs & trunk lasting 1 – 2 days
- laryngopharyngeal oedema
- sometimes abdominal pain (mesenteric oedema).

- The characteristic **sub-epithelial oedema** resulting from increased vascular permeability & leakage from post-capillary venules, is **due to bradykinin** produced by uninhibited **kallikrein**.
- **3 types of disease:**

- **type 1**: 85% of cases, the classic form. ↓ C1 INH mass & ↓ function.
- **type 2**: 15% of cases. C1 INH protein is secreted (mass levels may be normal or even ↑), but mutations in the active site disable its function.
- **acquired**: rare. Presents in adulthood. ? underlying lymphoma or myeloma. Due to ↑ **consumption** of C1INH rather than ↓ synthesis or function.

Q. Why are C4 & C3 automatically assayed with C1 INH?

A. To confirm C1 INH deficiency & sample preservation.

- ↓ **C4 confirms deficient C1 INH**, especially during acute angioedema, because it is a substrate of activated C1.
- C3 levels are relatively well preserved in C1 INH deficiency, thus ↓ **C3 suggests sample degradation** might have caused ↓ C4 & ↓ C1 INH results.

chloride (serum) 3d See profiles

Daily

See anion gap & bicarbonate.

98 – 107 mmol/L
Roche Cobas ISE indirect Na-K-Cl for Gen.2 V7

Use

- Serum Cl assay may be useful in puzzling acid/base disturbaces eg. to reveal abnormal **acid anions** by calculating the **anion gap** or in states where Cl & Na losses are not as equal as usually can be presumed eg. **pyloric stenosis**.

Background

- Chloride is the **principal anion countering sodium** cations.
- Thus, **Cl levels usually reflect sodium concentration** & assay does not provide new data.

chloride (sweat) 3d 50uL sweat. **Wescor Macroduct system** Daily

See Na (sweat) & immunoreactive trypsin.

Normal	< 40 mmol/L
Inconclusive	40 – 60
Consistent with CF	> 60
ACB 2003	

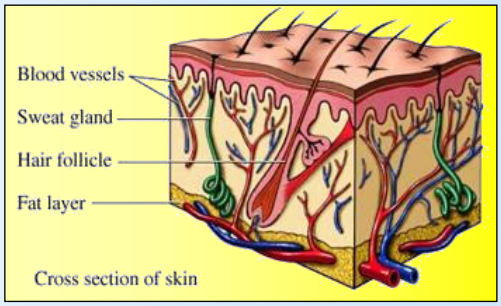
Use

- Ix of **cystic fibrosis**

Test

- Collection of sweat is fiddly & **experience** & **adherence to the *guidelines* are essential** for reliable results.
- Defer collection in patients who are:

- < 7d old
- < 3kg
- dehydrated
- systemically unwell
- or who have marked oedema or eczema.



Background

- Sweat **chloride concentration is ↑ in cystic fibrosis**
- This is the basis of sweat testing
- See the excellent: "*Guidelines for the performance of the sweat test for the investigation of cystic fibrosis in the UK*" ACB 2003

cholesterol (total) 3d See profiles

See acute phase, cholesterol:HDL ratio, HDL, LDL, **MRI** & Tg.

Daily

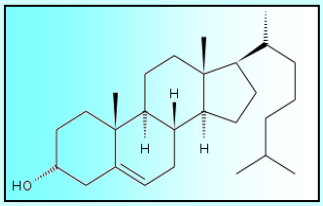
• **Fasting is inessential** except that Tg may be too high postprandially for calculation of LDL cholesterol.

Target levels.

1° prevention = ↓ risk of 1st stroke or onset of CHD**.**
2° prevention = ↓ risk of more.

		Tot. chol. (mmol/L)	**or LDL chol.** (mmol/L)
NICE	**Primary prevention**: No target given, even if on a statin for a 10y CVD risk >20%	No target	No target
	Secondary prevention	≤ 4.0 (at most ≤ 5.0)	≤ 2.0 (at most ≤ 3.0)
NICE CG67			
JBS2	**Primary prevention:** FH, DM, TC/ HDL ≥6.0, 10y CVD risk >20% etc.	≤ 4.0 (at most ≤5.0) or a 25% ↓	≤ 2.0 (at most ≤3.0) or a 30% ↓
	Secondary prevention	≤ 4.0 (at most ≤ 5.0) or a 25%	≤ 2.0 (at most ≤3.0) or a 30% ↓
JBS 2005			

Background A link between extreme hypercholesterolaemia, extravascular deposits eg. tendon xanthomata & premature atheromatous disease, has been known for a long time. But it was not until **1961** that the link between plasma cholesterol levels in the general population & the risk of *coronary heart disease* (CHD), was objectively demonstrated by the **Framingham Heart Study** (FHS). This followed healthy people, possible risk factors & the occurrence of CHD over the years from 1948. It is still going & now includes grandchildren.



Recent guidelines for 1y & 2ndy prevention, **combine the risks of CHD** & **strokes** in the term **cardiovascular disease (CVD) risk** ie. CHD risk + stroke risk = CVD risk. A CVD risk of 20 % in 10y, approx. = CHD risk of 15% in 10y.

Components of total cholesterol Serum **total cholesterol** = cholesterol in **low density lipoprotein** (LDL) particles, which is "going" to tissues (**forward transport**) & is positively correlated with CVD risk **plus** cholesterol in **high density lipoprotein** (HDL) particles which is "returning" to the liver (**reverse transport**) & is protective (FHS 1988). This sum is actually less than the assayed total because some cholesterol is present in other lipid particles, notably VLDL & IDL (see LDL figure).

A marker of CVD risk Population studies show a + ve correlation between serum total cholesterol & CHD mortality, but it is an imprecise indicator of risk. In 2 people with the same total cholesterol level, one with much of it as HDL would have a low CHD risk, whereas if the other had little of it as HDL, their risk might be much ↑. These opposing effects are taken account of by using the total cholesterol/HDL ratio to calculate CVD risk, rather than absolute levels of either.

This ratio is only one of **several factors** eg. BP & smoking, which **affect the CVD risk**, which is the "bottom line" in deciding the need for therapy, especially in primary prevention. For more details, see the *Joint British Societies' Guidelines (JBS2) on the prevention of cardiovascular disease* (JBS 2005) & the *BNF*.

Causes of ↑ • familial & polygenic hyperlipoproteinaemia I, IIa, IIb, III, IV & V • hyper-α-lipoproteinaemia • cholestasis • hepatocellular disease • nephrotic syn. • CRF • hypothyroidism • metabolic syn. • alcoholism • diabetes mellitus • glycogen storage disease types I, III &VI • anorexia nervosa • pregnancy.

Causes of ↓ • hypo-α-lipoproteinaemia (Tangier disease) • hypo & abetalipoproteinaemia • severe acute illness • malnutrition • malabsorption • hepatic necrosis.

cholesterol (total):HDL ratio 3d See profiles

Daily

Desirable ≤ 6
JBS guidelines 2005

See cholesterol (total), HDL, LDL & Tg.

Use

- Estimation of the net contribution of cholesterol to **CVD risk**.

Background

- Serum total cholesterol (TC) correlates +vely with the risk of atherosclerotic cardiovascular disease (CVD).
- TC's low density lipoprotein cholesterol fraction represents **forward transport** of cholesterol to tissues eg. arterial walls, & correlates +vely with CVD risk, but its use as a risk marker is impaired by **assay difficulties**.
- In contrast, cholesterol in high density lipoprotein particles (HDLC) is largely moving from tissues back to the liver (**reverse transport**) where it can be disposed of in bile. Serum levels correlate – vely with CVD risk.
- The **TC:HDLC ratio** takes account of these opposing factors, in a practicable way, for more accurate CVD risk prediction than when either is used alone.
- The **full CVD risk** is obtained by combining the TC:HDLC ratio with **other factors** eg. BP & smoking.

See

- Joint British Societies' Guidelines (JBS2) on the prevention of cardiovascular disease. JBS 2005.
- Lipid modification – cardiovascular risk assessment & the modification of blood lipids for the primary & secondary prevention of CVD. NICE CG67
- BNF.

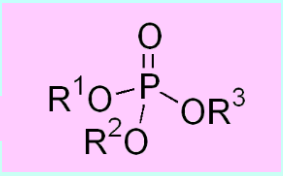
cholinesterase (RBC) 0d 1.0 mL
See serum cholinesterase.

Sent

Reference sent with result
Health & Safety Lab. Buxton

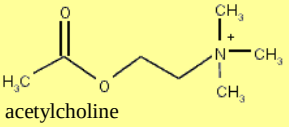
Use • Ix of **organophosphate poisoning**

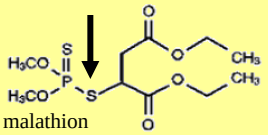
Background



• Organophosphate is a **catch-all term for phosphoric acid esters** eg. includes DNA

• Usually refers to substituted derivatives used as insecticides eg. malathion, which **inhibit *acetylcholinesterase*** (AChE).


acetylcholine


malathion

• AChE is located in **synaptic clefts & neuromuscular junctions**.
• AChE **terminates the action of the neurotransmitter acetylcholine** by cleaving the ester link between its acetic acid & choline components.
• The toxicity of organophosphates depends on their **particular physicochemical properties** & **species specific metabolism** eg. malathion toxicity is ↑↑ by conversion to malaoxon by oxidative P450 enzyme in insects.
• **High AChE activity is essential**. Organophosphates impede this by covalently bonding to the enzyme's active site eg. AChE cleavage of the thioester of malathion at the point shown by the arrow, leaves the phosphoric acid moiety bonded to an essential serine residue in the active site, preventing any further ester hydrolysis.
• Loss of AChE activity causes persistent stimulation of ACh signalling in autonomic & central nerves & at the neuromuscular junction in voluntary muscle (initially contraction, then flaccid paralysis *cf.* suxamethonium).
• **Plasma cholinesterase is affected too** but loss of its activity is not life threatening & the prevalence of variant forms with ↓ activity make it a less than ideal index of poisoning.
• Fortunately, **AChE activity in red blood cells mirrors activity in nerves** (inaccessible for assay).

• **Acute toxicity**. **Sludgem** & the **3 Bears** due to ↑↑ parasympathetic activity:

- **S**alivation & sweating
- **L**acrimation
- **U**rination
- **D**efaecation
- **G**ut eg. groans & diarrhoea
- **E**mesis (vomiting)
- **M**iosis (small pupils)

The 3 Bears:
- **B**ronchospasm
- **B**ronchorrhoea
- **B**radycardia

*Epilepsy
*Muscle fasciculation/weakness
*Mental eg. restless, dizzy

* not parasympathetic effects but conveniently linked to the mnemonic.

• **Long term effects** & the consequences of **chronic low level exposure** are disputed but neuropathies & psychiatric conditions have been reported. RBC cholinesterase results are normal except in on-going exposure.

cholinesterase (serum) 3d 1.5mL

Sent

600 – 1400 IU/L
Lewisham Hospital

See RBC cholinesterase & acetylcholine receptor Abs

Use • Ix of **"suxamethonium apnoea"** in patients & relatives.

Test

Step 1 Cholinesterase activity in serum is assayed.
Step 2 If activity is below the ref. range median, further assays (**phenotyping**) are performed to identify the enzyme forms present by their characteristic vulnerabilities to inhibitors.

Background

- Synthesised by the **liver**, serum/plasma cholinesterase is distinct from the more familiar *acetyl cholinesterase* in synapses & motor-endplates, which is responsible for inactivating the neurotransmitter acetylcholine.
- **Variant alleles** exist, which cause partial to total loss of function.
- Deficient activity **prolongs the action of drugs which are inactivated by ester hydrolysis** by this enzyme eg. the muscle relaxants **suxamethonium** & **mivacurium**. Most cases present this way.
- **Natural role unclear**.

Causes of ↓

- congenital
- normal pregnancy.
- liver disease
- burns & other severe illnesses.

} May add to effect of minor congenital deficiency & make it clinicaly significant.

Family studies

- The **first degree relatives** (mum, dad, sibs, kids) of people found to be deficient, should be studied too. This is best done by sending **clotted blood** for enzymology & **EDTA whole blood** in a purple tube for DNA analysis, from each relative.
- **Caution,** family studies (even without DNA analysis) can uncover paternity issues.

chromium 3d 3mL

Sent

See cobalt & insulin.

Health, no exposure.	< 10.0 nmol/L (0.5 ug/L)
Joint wear. MHRA guide figure	< 135.0 .. (7.0)
Functioning **metal-on-plastic** hip	15.0 *median* (0.8)
.. x1 **metal-on-metal** hip	45.0 .. (2.3)
.. x2 **metal-on-metal** hips	45.0 .. (2.3)
Charing Cross Hospital [nmol/L x 0.052 = ug/L]	

Use

- Monitoring wear of **Metal on Metal** hip joints. See cobalt for details.
- Ix nutrition, but see below

Background

- Body stores of this **essential** trace element are limited, but ordinarily, deficiency does not arise because chromium is **abundant** in the environment.
- Patients on TPN can show evidence of deficiency (insulin resistance & neuropathy) if **trace element supplements** are not given.
- **A poor index of deficiency**, because the level associated with this is below the assay detection limit. Thus, TPN supplements are given **prophylactically**.

Occupational testing

- **The lab. cannot accept occupational screening samples.**
- It does not operate the necessary written chain-of-custody required to prove ownership of results beyond dispute.
- Assays for monitoring occupational exposure are **mainly based on urine**.

chromogranin A (CgA) 0d 1.5mL

Sent

See ACTH, aldosterone, CgB, C-peptide, GAGs, gut hormone profile, 5HIAA, I cell dis., insulin, renin & VMA.

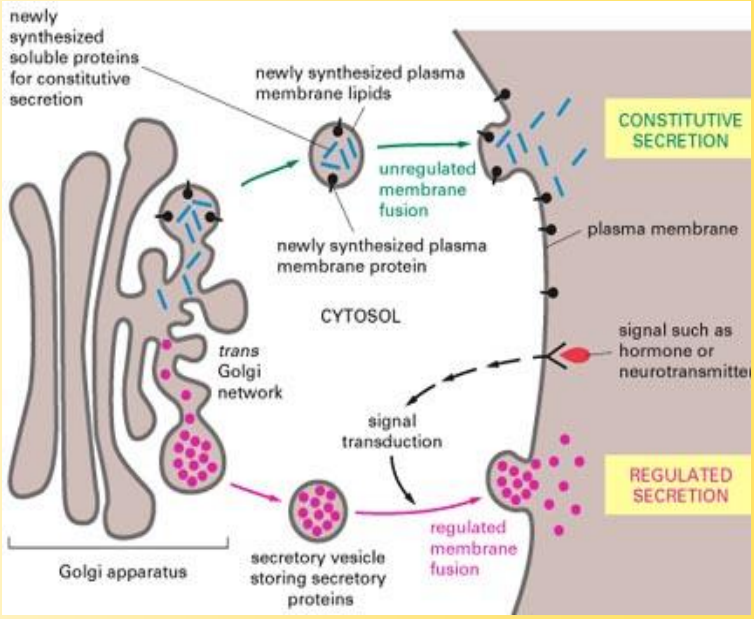
≤ 6.0 nmol/L (0 – 4 before 17/12/09)
Sheffield Protein Reference Unit

Use

- Detection of **carcinoids** & **phaeochromocytomas**.
- Monitoring **recurrence** & **response to therapy** of these & other endocrine neoplasms.

Background

- A **100 kDa protein** in all **regulated secretory vesicles** (RSV) of neural, endocrine & neuroendocrine tissues, which is secreted with the contents.
- **Function is unclear** & **independent of** the characteristic **secretion of the tissue**: its role may be to sort & concentrate molecules in RSVs in preparation for exocytosis.
- It may be a **prohormone** for bioactive peptides eg. vasostatin.
- CgA is **more sensitive** (80%) but less specific than 5HIAA & "VMA" ie. **CgA is useful for follow-up**.
- **Screening** normal people with CgA is only for when risk of neuroendocrine neoplasia is high eg. families with **MEN** or **von Hippel-Lindau** syn.
- The related peptide, **chromogranin B** **is less sensitive** than CgA for tumour detection but it is **more specific** because its levels rise less than those of CgA with eg. PPIs & renal impairment. Thus, it is a **complement to CgA** rather than an assay of independent value.
- In **non-secretory endocrine neoplasia**, CgA secretion may still be present.
- CgA levels are unaffected by **age, gender or pregnancy**.



Neoplastic causes of ↑

- phaeochromocytoma
- parathyroid adenoma
- SCC lung
- medullary thyroid carcinoma
- carcinoid tumour
- carotid body tumour
- islet cell adenoma/carcinoma
- pituitary adenoma
- neuroblastoma

Non-neoplastic causes of ↑

- essential hypertension
- ↓ GFR (can be as high as with tumours)
- PPIs

chromogranin B (CgB) 0d 1.5mL

Sent

(syn. secretogranin 1).
See CgA & gut hormone profile.

Reference sent with result
Sheffield Protein Reference Unit

Use

- For **detection** of **neuroendocrine tumours** (NETs) eg. carcinoids & phaeochromocytomas
- For **monitoring** recurrence & response to therapy.

Background

- A **110 kDa protein** in the **regulated secretory vesicles** (RSV) of neural, endocrine & neuroendocrine tissues, which is secreted with the contents ie. it is like CgA.
- **Its function is unclear** & **independent of the characteristic secretion of the tissue**. It may sort & concentrate molecules in RSVs in preparation for exocytosis, again like CgA.
- It may also be a **prohormone** for bioactive peptides eg. secretolytin & GAWK
- **CgB** is **less sensitive than CgA** for tumour detection but it is **more specific** because its levels rise less with eg. PPIs & renal impairment, than those of CgA ie. it **complements CgA** rather than replaces it.
- Levels are unaffected by age, gender or pregnancy.

Neoplastic causes of ↑

- phaeochromocytoma
- parathyroid adenoma
- SCC lung
- medullary thyroid carcinoma
- carcinoid tumour
- carotid body tumour
- islet cell adenoma/carcinoma
- pituitary adenoma
- neuroblastoma

Non-neoplastic causes of ↑

- essential hypertension
- ↓ GFR (can be as high as with tumours)
- PPIs

clobazam 3d 1.5mL **No gel**

Sent

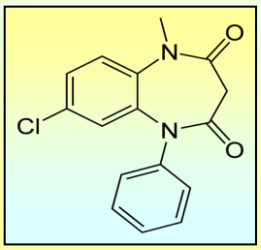
See therapeutic drug monitoring.

- **Pre-dose sample.**

Clobazam	< 200 ug/L
Desmethylclobazam	< 2000
St Thomas' Hospital.	

Use **Ix:**

- toxicity or non-compliance
- failure to establish or maintain therapeutic effect.



Background

- An anxiolytic & anticonvulsant benzodiazepine derivative
- **Demethylation** in the liver produces the **active metabolite N-desmethylclobazam** which like clobazam, enhances GABA activated chloride currents at $GABA_A$ receptor coupled chloride channels, resulting in inhibitory effects on the CNS.

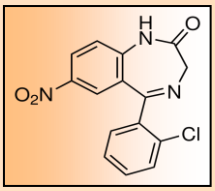
clonazepam 3d 1.5mL

Sent

See therapeutic drug monitoring.

- **Pre-dose sample.**

25 – 85 ug/L
St.Thomas' Hospital



Use Ix:

- toxicity or non-compliance
- failure to establish or maintain therapeutic effect.

Background

- Clonazepam acts on **benzodiazepine receptors** to enhance the binding of GABA to $GABA_A$ receptors, which results in inhibitory effects on the CNS.
- **Anxiolytic & anticonvulsant**, but drowsiness may be a problem.
- Clearance is by **hepatic metabolism** followed by renal excretion of the products.

clozapine 3d 3mL

Sent

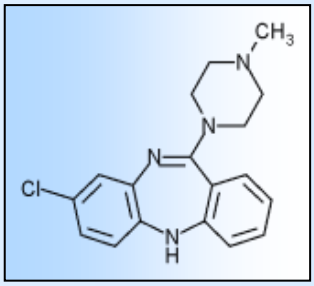
See olanzapine & TDM.

- **Pre-dose** sample.
- **Drug monitoring not needed routinely.**
- Monitor with **FBC** & **LFT**.

Clozapine	0.35 – 0.5 mg/L
Norclozapine	70 % of clozapine level if compliant
Kings College Hospital	

Use Ix of:

- toxicity
- inexplicable failure to establish or maintain therapeutic effect eg. non-compliance.



References

- A therapeutic **reference range** has not been clearly established, but:

< 0.1 mg/L	Unlikely to be therapeutic.
0.35 – 0.5	Likely to treat refractory schizophrenia
> 0.5	↑ risk of convulsions.

Background & interpretation

- Clozapine was the 1st **atypical antipsychotic** drug. **Serious side-effects** eg. agranulocytosis, cardiomyopathy & Seizures occur, but it can treat schizophrenia resistant to other drugs.
- **Agranulocytosis-risk** is monitored with regular **FBC,** not drug assay.
- **Norclozapine**, a metabolite of clozapine, can be useful to assay in the Ix of compliance. On stable therapy, the level is approx. **70 %** of clozapine's. A ↓ fraction suggests *recently* ↑ clozapine intake.
- Clozapine **clearance is induced** by the drug & **smoking**.
- **Smoking cessation** can cause toxic concentrations to develop on a previously satisfactory dose.
- **Equilibration** takes approx. 4 d after a dose-change.

cobalt 3d 3mL

See chromium, vit B12 & workload.

Sent

Health, no exposure.	< 10.0 nmol/L (0.59 ug/L)
Joint wear. MHRA guide figure	< 120.0 (7.0)
Functioning **metal-on-plastic** hip	10.0 *median* (0.6)
.. x1 **metal-on-metal** hip	30.0 .. (1.7)
.. x2	45.0 .. (2.4)
Charing Cross Hospital	[nmol/L x 0.059 = ug/L]

Use

- Monitoring wear of **METAL on METAL** joints.
- Ix **nutrition –** rarely necessary.

Background

- An **essential** element, it is present in eg. vitamin B12 (cobalamin).
- Illness due to deficiency is extremely rare in humans.
- However, prophylactic supplements are given with TPN, as with chromium.

The Medicines & Healthcare Products Regulatory Agency in April 2010 (updated June 2012, see MHRA 2012) gave advice on monitoring wear of metal on metal hip replacements with plasma cobalt and chromium measurements:

• Investigate patients with painful MoM hip replacements. Specific tests should include evaluation of cobalt and chromium ion levels in the patient's blood and cross sectional imaging including MRI or ultrasound scan

• Consider measuring cobalt and chromium ion levels in the blood and/or cross sectional imaging for the following patient groups:
- patients with radiological features associated with adverse outcomes including component position
- patients with small component size (hip resurfacing arthroplasty only)
- cases where the patient or surgeon is concerned about the MoM hip replacement
- cohorts of patients where there is concern about higher than expected rates of failure

• If either cobalt or chromium ion levels are elevated above seven parts per billion (**120 nmol/L cobalt**,**135 nmol/L chromium**), then a second test should be performed three months after the first in order to identify patients who require closer surveillance, which may include cross sectional imaging

Complement C3 3d 1.5mL
See acute-phase response, C4, CRP, & C1 esterase inhibitor.

Daily

0.9 – 1.8 g/L
Roche Cobas C3C-2 method sheet V7

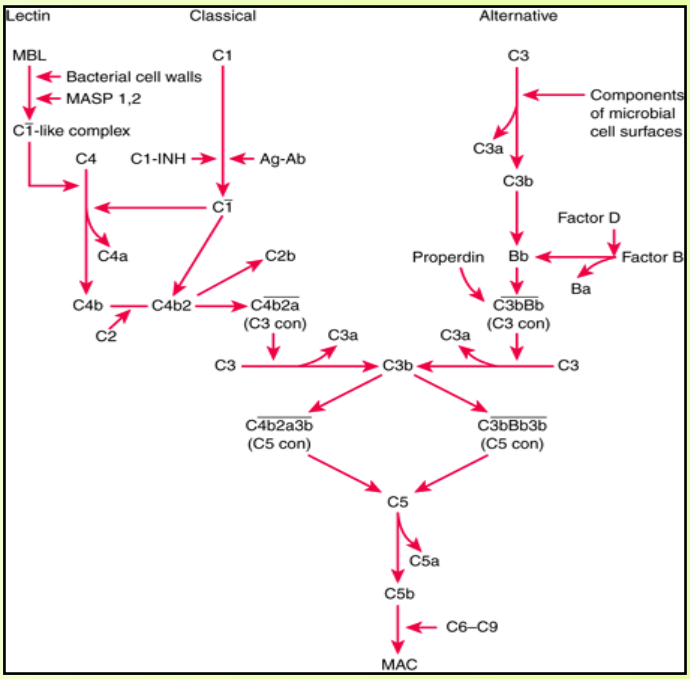
Use

• Ix of immune-mediated diseases eg. Rheumatoid dis. & SLE Assay C4 too to ↑ understanding & clarity.

Background

• **C3** results reflect activation of **all 3** complement activation routes. C1 esterase inhibitor deficiency effects are reflected in C3 (& C4) levels:

	Classical & lectin route	**Alt. route**	**C1 INH def.**
C3	↓	↓	normal
C4	↓	normal	↓



• Complement proteins & peptide factors form the **humoral innate immune system**, which destroys (or augments the elimination of) bacteria, viruses, parasites, cells & membranes without cellular effectors or immune memory. 25 complement proteins in plasma are synthesized by the **liver** eg.
enzymes, activators, inhibitors & effectors.

• Some proteins have subunits with distinct functions eg. **C3**, the most abundant complement protein, when activated is cleaved to form**:**

- **C3a** (anaphylatoxin): → mast cell degranulation
- **C3b** (opsonin): binds bacteria → phagocytosis
- **C3c** & **C3d**: equimolar fragments.

• **Activation of C3 causes its level to ↓** but the level of downstream fragments may ↑ eg. C3c & C3d.

• Sequential complement activation causes **amplification**, **signalling** (eg. to platelets, **mast** cells & neutrophils), **opsoninisation** & assembly of the **membrane attack complex** - a pore-like protein which lyses bacteria.

• There are **3 routes** to activation of complement ie. the production of C3 convertase (C3 con in diagram)**:**

- **Classical** route: Ag/Ab (IgM & IgG) complexes on pathogen surfaces or complexed CRP, activate the zymogen form (inactive enzyme precursor) of C1.
- **Alternative** route: activated by bacterial lipopolysaccharide & other endotoxins.
- **Mannose binding lectin** (MBL) route: binding of the plasma protein **lectin** to mannose residues on bacteria.

Causes of ↓

• sample degradation
• acute phase response
• SLE
• Rheumatoid disease
• sepsis
• SBE
• membranoproliferative glomerulonephtitis
• parasites
• viraemia
• hepatic failure
• congenital deficiency

Causes of ↑ (< 2 fold, but can be sufficient to mask other conditions which would lower it)

• pregnancy
• old sample
• acute phase response (stimulates synthesis, biphasic, see ↓ above) .

complement C4 3d 1.5mL

Daily

See acute-phase response, C1 esterase inibitor, C3 & CRP.

0.1 – 0.4 g/L
Roche Cobas C4-2 method sheet V8

Use

- Diagnosis & monitoring immune-mediated diseases eg. Rh & SLE. .
- Assay C3 too to ↑ understanding & clarity.

Background

See C3 for further info. & DIAGRAM.

- Complement C4 is activated by 2 routes.
- **classical route**: Ag/Ab (IgM & IgG) complexes on pathogen surfaces or complexed CRP, activate the zymogen form (inactive enzyme precursor) of C1.
- **mannose binding lectin** (MBL) route: binding of the plasma protein **lectin** to mannose residues on bacteria.
- As with C3, **consumption of C4** proteins by activation can ↓ the serum level.
- This is **countered by ↑ synthesis** by the liver, such that the **C4 level may be deceptively normal** or even ↑ despite vigorous disease activity, especially **in acute inflammation** or trauma.
- In more **moderate or chronic inflammation**, the synthetic response is weaker & **C4 levels ↓.**

Causes of ↑

- ↑ synthesis in the acute phase response.

Causes of ↓

- ↑ consumption with inadequate synthesis to prevent ↓ eg. SLE, Rh dis., vasculitis & nephritis
- Acquired deficiency due to ↓ synthesis eg. liver failure.
- Carriers of the C4 null-allele (2.5% of general pop.)
- C4 is undetectable in patients who are homozygous for these null-alleles.

copper (plasma) 3d 3mL

Sent

See caeruloplasmin, Cu (urine) & **MRI**.

0 - 4 m	1.4 - 7.2 umol/L
4 - 6 m	3.9 - 17.3
7 - 12m	7.9 - 20.5
> 1y & adults	11 - 20
Pregnancy, 16/40 to term	27 - 40
Wilson's disease	< 4
Royal Surrey County Hospital	

Causes of ↓

- see caeruloplasmin

Causes of ↑

- ↑ with age
- pregnancy
- drugs: oestrogen eg. HRT & OCP, carbamazepine, phenytoin, phenobarbitone.
- inflammation
- biliary cirrhosis
- leukaemia, lymphoma
- hyper & hypothyroidism
- cancer: gi tract, bronchus, breast, cervix, bone, haematopoietic

copper (24h urine) 3d 24h urine

Sent

See caeruloplasmin & copper (plasma).

• **Procedure**: Urinate in the toilet & note the time (can be any time). Collect all urine for the next 24h. The last one to collect is whatever can be passed at the noted-time next day.

Normal diet	Adults	< 0.95 umol/24h
	Wilson's dis.	> 1.6
Post-penicillamine challenge (500mg po. before & mid 24h urine collection)	Adults	< 12
	Wilson's dis.	> 25
Royal Surrey County Hospital		

Causes of ↑

- Wilson's disease (can be normal)
- **Cholestasis of any cause**

cortisol (serum) 3d 1.5mL

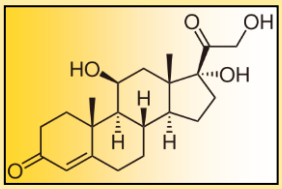
Daily

See ACTH, aldosterone, comments, cortisol (urine), 11-deoxycortisol, K, glucagon stim. test, 17-OHP, ONDEX, renin & SST.

7 - 10 am	171 - 536 nmol/L
4 - 8 pm	64 - 327 *(5th - 95th cent)*
Roche Cobas Cortisol method sheet V19	

• Take blood at **9am ideally**, unless it's part of a profile or dynamic test.

Background



- Interpretations based on single measurements are weakened by the pulsatility, diurnal rhythm & response to stress of cortisol secretion, especially at times other than when levels are at their physiological peak at **8 – 9 am**.
- Prednisolone & methylprednisolone **cross-react & ↑ results**
- Pathological & normal levels **overlap** ++.
- Out-of-range results should be **confirmed** or better still, investigated with a dynamic test eg. a **dexamethasone suppression test** for suspected hypersecretion or a **short Synacthen test** for deficiency.

cortisol (urine) 3d 24h urine

Sent

See cortisol (serum), 5 day dex suppr test & ONDEX.

25 – 280 nmol/24h
St Helier Hospital

• **Procedure**: Urinate in the toilet & note the time (can be any time). Collect all urine for the next 24h. The last one to collect is whatever can be passed at the noted-time next day.

Background

- An alternative to serum cortisol assays & dex. suppr. tests for **Ix Cushing's disease**.
- However, the difficulty patients have with collecting a true 24h specimen diminishes the reliability of results.
- Urine is **less useful for investigating Addison's disease** because of technical problems with the low levels of cortisol & their less than clear separation from values in health. The Synacthen test is better.

C-peptide 0d 1.5mL

Hypoglycaemia is essential for clear interpretation.

Sent

Result & interpretation
Royal Surrey County Hospital

Hypoglycaemia is crucial to interpretation	
< 60 y old	plasma glucose ≤ 2.0 mmol/L
> 60 y	.. .. ≤ 2.5

See β hydroxybutyrate, carnitine, CgA, comments, cystine, FFA, glucose, insulin & sulphonylurea.

Use

- Ix **hypoglycaemia**.
- **Classification** of the type of diabetes mellitus, especially in children.
  For a better result & interpretation, give **clinical details** & measure **glucose** & **insulin** too.

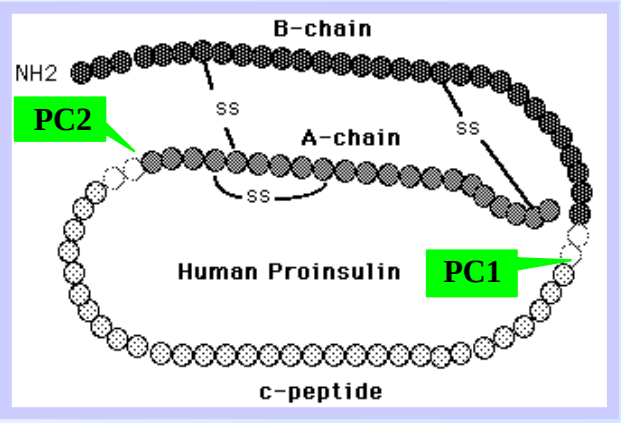
Background

- C-peptide is a 31 amino acids long **sequence in proinsulin**, the insulin precursor, between C & N-terminal domains which become the A & B chains of insulin, respectively.
- Proinsulin, synthesised in the ER, moves via the Golgi to the regulated secretory vesicles (see chromogranin A) where two enzymes (proprotein **convertases** 1 & 2) cleave it to release C-peptide & form insulin.
- C-peptide is "scaffolding" for the correct **alignment** & assembly of the A & B chains into insulin.
- Does it have **bioactivity?** Circumstantial clinical evidence & lab. studies suggest that it may ↓ diabetic complications eg. glomerular hyperfiltration & albuminuria. However, unlike insulin, the C-peptide amino acid **sequence is poorly conserved** across species & the **receptor is unknown**. C-peptide is **probably just an efficient way to synthesise insulin**.
- The short plasma half-life of insulin (4 min.) compared with that of C-peptide (30 min.) means that despite **equimolar** secretion, the **molar conc. of C-peptide in plasma is approx. 5x that of insulin**.
- C-peptide assay can shed light on the **source of insulin** in plasma during **hypoglycaemia:**
- **exogenous** insulin preparations do not contain C-peptide ie. plasma insulin will be ↑ & C-peptide ↓.
- in **endogenous** secretion due to eg. sulphonylurea drugs or insulinoma – both insulin & C-peptide will be ↑.
- Some consider C-peptide to be a superior marker of insulin hypersecretion than insulin itself, because of its slower dynamics.

Causes of ↑

- ↑ endogenous insulin secretion
  eg. sulphonyluria, insulinoma, insulin resistance.
- Food
- ↓ GFR (C-peptide clearance is 90% renal)

In β cell secretory granules, PC1 (proprotein convertase 1) cleaves proinsulin at the C-peptide N-terminus, which prepares the way for a 2nd convertase (PC2) to cleave at the C-terminus & release C-peptide & insulin.



creatine kinase (CK) 3d 1.5mL

Daily

See CDT, comments, creatinine, cTnT & ethanol.

Women	26 – 192 U/L
Men	39 – 308
Roche Cobas CK method sheet V3 (5th – 95th cent)	

Use

- A **non-specific marker of muscle** injury/disease.

Background

- This **muscle-enzyme** catalyses the transfer of high-energy phosphate from stored phosphocreatine to ADP to rapidly **replenish ATP** consumed by muscle use (see figure).
- The bulk of serum total CK activity comes from **skeletal muscle**, but the level can ↑ with release from any muscle since all muscles contain **isoforms** of creatine kinase in different proportions eg. BB & MB.
- CK **isoform study is no longer available**, because the principal use was detection of MI & this is now done better by the much **more specific cardiac-marker**, **cTnT** (cardiac troponin T).
- In **MI**, serum CK starts rising approx. 4h after chest pain begins, peaks at 24h & falls to basal in 48h.

Causes of ↑ CK

- Muscle injury eg. vigorous exercise, trauma, surgery, MI, grandmal convulsions, cardioversion etc.
- Myositis, myocarditis, dermatomyositis
- Muscular dystrophy
- Statins
- Alcoholism
- Hypothyroidism
- Carcinoma – especially prostate, bladder & GI tract
- Pregnancy

Causes of ↓ CK (little significance)

- ↓ muscle mass
- sedentary lifestyle
- bedrest without crushing.

creatinine (serum) 3d See profiles Daily

see ACR, Al, CK, comments, creat. (24h U), creat. clear., creat. (U conc.)

There are 2 assay methods: eGFR, K, **MRI**, Na, PO_4 & urea.

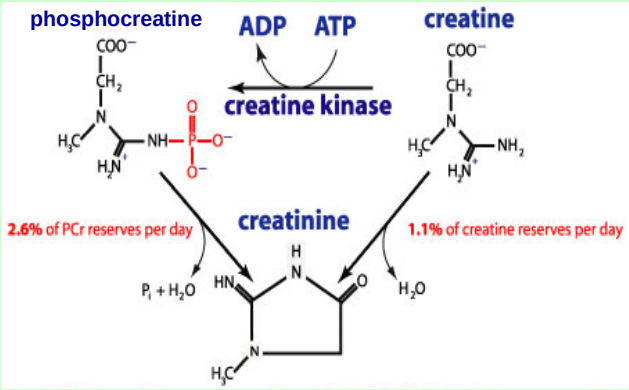
1. Jaffé assay.

Inexpensive but ↑ bilirubin interferes & ↓ the result

Women	44 – 80 umol/L
Men	62 – 106
Roche Cobas CREJ2 method sheet V7	

2. Enzymatic assay.

No bilirubin interference, but costly.
For adults who have ↑↑ bilirubin & **all children**.



Children	premature	29 - 87 umol/L
	1 - 14d	27 - 77
	2 - 12m	14 - 34
	1 - < 3y	15 - 31
	3 - < 5y	23 - 37
	5 - < 7y	25 - 42
	7 - < 9y	30 - 47
	9 - < 11y	29 - 56
	11 - < 13y	39 - 60
	13 - < 15y	40 - 68
Adults	**Men**	59 - 104
	Women	45 - 84 umol/L
Roche Cobas CREP2 method sheet V5		

Background

- Each day, 2% of the **creatine** in muscle is **spontaneously converted to creatinine**, the total quantity being dependent on the total muscle mass.
- **Urinary excretion** is thus fairly **constant** & can be used as a marker of time to reduce the confounding effect of dilution on results of other substances in urine eg. albumin/creatinine ratio.
- The **non-linear relationship between serum creatinine & GFR** (glomerular filtration rate) makes serum creatinine an insensitive marker of early renal impairment.
- Estimation of GFR from serum creatinine offers some improvement.

Causes of ↑

- ↓ GFR
- a large meat intake (even if GFR is normal)
- ↑ muscle mass eg. body building
- growth hormone excess eg. acromegaly
- hyperthyroidism

Causes of ↓

- ↓ muscle mass eg. aging, wasting
- pregnancy, especially in 1st & 2nd trimesters

Serum creatinine or urea for gauging renal function?

- They are not equivalent & both have serious failings, but they are cheap to measure & trends in results over time can be useful. • Always interpret results in relation to previous results, therapy & clinical factors.

Urea

- more influenced by diet & tissue breakdown (exaggerated by ↓ GFR)
- passive reabsorption of urea from tubular fluid (>40%) makes serum urea a poor index of GFR ie. it is higher than the GFR would suggest.

Creatinine

- serum creatinine may look deceptively normal, despite ↓ GFR, especially if there is ↓ production due to muscle wasting eg. **elderly people**.
- tubular secretion ↑ as the serum level ↑ with ↓ GFR, which further ↓ the sensitivity of serum creatinine as a marker of ↓ GFR ie. it is lower than the GFR would suggest.

creatinine (24h urine) 3d 24h urine

Daily

See creat. clearance, creat. (serum), & creat. (U conc.), eGFR

Jaffe method.

Women	7.0 – 14.0 mmol/24h
Men	9.0 – 21.0
Roche Cobas CREJ2 method sheet V7	

Use

- **Not especially useful** on its own.
- However, this assay**:**

- provides an indication of **under or over collection of 24h urine samples** for another test eg. 5HIAA
- is part of the measurement of **creatinine clearance**. But see eGFR.

creatinine (urine conc.) 3d 20mL urine

Daily

See creatinine (serum), creatinine (24h urine), eGFR & urea (urine conc).

- **Jaffe method.**

Women	2470 – 19200 umol/L (early am)
Men	3450 – 22900
Roche Cobas CREJ2 method sheet V7	

Use

- **Not very useful in isolation**, because of considerable **variation with dilution**.
- To correct for the effect of dilution on other analytes by expression of them as a ratio eg. ACR
- To answer the question, **could there be urine in this drain fluid?**

- Urine creatinine conc. is normally 20 – 150 x the serum or tissue fluid conc.
- **NB**. Small urine “leaks” may elevate the drain fluid creatinine conc. less distinctly.

creatinine clearance 3d

See creat. (serum), creat. (24h U), eGFR, urea (24h U) & urea (serum).

PLUS

Daily

24h urine 1.5mL

- **Take blood for creatinine within 1 d of the urine.**
- **Preparation.** Patients should be well hydrated, physiologically stable & without acute illness. Heavy exercise & gross consumption of tea, coffee & cigarettes, should be avoided during the urine collection.
- **Procedure**: Urinate in the toilet & note the time (can be any time). Collect all urine for the next 24h. The last to collect is whatever can be passed at the noted-time next day.

0 – 1y old		72 mL/min./1.73 m^2
1y		45
2y		55
3y		60
4y		71
5y		73
6y		64
7y		67
8y		72
9y		83
10y		89
11y		92
12y		109
13 – 14y		86
20 – 29y	**Women**	72 - 110
	Men	94 - 140
30 – 39y	**Women**	71 - 121
	Men	59 - 137
Each decade later, clearance falls 6.5 mL/min.		
Tietz		

Use

- Measurement of GFR. **Superceded by eGFR.**

Background

- A key measure of renal function is the glomerular filtration rate (**GFR**), which is the total volume of liquid entering the renal tubules each minute.
- If on passage through the tubule, a solute in this filtrate is neither reabsorbed nor added to, the quantity passed out in urine/minute is the same as the quantity filtered by the glomeruli/minute.
- Since the serum conc. of the freely filtered solute is known & the same as in the filtrate, it is simple arithmetic to calculate the volume of filtrate which would contain the quantity of solute excreted per minute ie. the GFR:

Filtrate vol/min (GFR, mL) = **quantity in urine/min** (units/min) ÷ **plasma conc** (units/mL)

- **In reality**, there are no endogenous solutes that are not reabsorbed or secreted by the tubule, to some degree. Thus the filtered solute load cannot be fully known from urine.
- Creatinine clearance **exaggerates GFR** because the filtered solute is supplemented by **creatinine secreted by tubules.** However, bias in the Jaffe assay of serum creatinine conveniently compensates & the creatinine clearance & GFR are not too dissimilar to be useable.
- **Inaccuracy of the 24 h urine collection** is the biggest error. It degrades creatinine clearance as a measure of GFR so much that **estimation from serum creatinine (eGFR) is now recommended**.

CRP (C reactive protein) 3d 1.5mL

Daily

≤ 5.0 mg/L
Roche Cobas CRPL3 method sheet V6

See ACE, acute phase response, C1 INH, C3, C4, ferritin, iron, **MRI** & Zn.

***Rough* clinical correlation**	
• Mild inflammation, severe viral infection	10 - 40 mg/L
• Significant inflammation, bacterial infection	40 - 200
• More severe state eg. burns & sepsis	> 200

Use

- Ix the presence of **inflammation** eg. bacterial infection, especially if clinical evidence is unclear eg. in:
  - acute on chronic inflammatory diseases such as Rh & IBD
  - babies/children
  - elderly
  - immunocompromised patients
  - peritoneal dialysis
  - SLE
  - neoplasia.
- **Clear sepsis does not need CRP** assay eg. an abscess.

Background

- A 120 kDa homopentameric ring protein.
- The **main marker of the acute phase response** & highly conserved in the animal kingdom.
- **Synthesised in the liver** in response to **IL-1**, serum levels ↑ **within 6h of injury/inflammation**.
- **Binds damaged cell membranes** & **bacterial components**, causing precipitation & complement activation, which results in inflammation, opsonisation & phagocytosis of CRP-bearing cell debris & bacteria.
- The response to **viruses** is generally less than to **bacteria**.
- Used *cautiously*, this enables CRP to contribute to predicting the causative agent.
- CAUTION. A –ve CRP result can be a **false negative** eg. in:
  - chronic inflammation or local disease.
  - neonates, even to bacteria.
- **CHD risk cannot be determined reliably from individual CRP results** ie. inter & intraindividual variation of CRP are much greater than the increment predicting cardiac risk, which requires population studies to detect it.

cryoglobulins 0d 4ml kept at 37°C

Daily

Interpretation

See BJP, free light chains & serum protein electrophoresis.

- **Before taking samples, contact lab.**

Test

- The blood sample MUST be **prevented from cooling** before it reaches the lab. Contact Pathology Reception for details of equipment & procedures for keeping samples at 37°C.
- In the lab., **serum protein electrophoresis** is run with **an aliquot kept at 37°C** & **another kept in the fridge**, which will lack proteins that have precipitated in the cold ie. cryoglobulins.

Background

- Abnormal proteins or complexes eg. in myeloma, can be **insoluble in plasma at temperatures below 37° C**. These occur in peripheral tissues eg. fingers, where **precipitation** can cause **Raynaud's** disease.

CSF oligoclonal bands 3d

See CSF protein & serum protein electrophoresis.

1.5mL blood

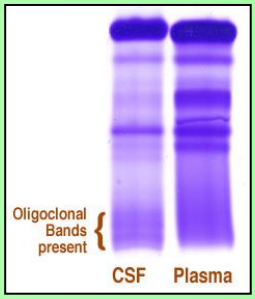
PLUS

Sent

1mL CSF

Comment
National Hospital for Neurology & Neurosurgery

Use • Diagnosis of MS



Background

- This test for **demyelinating diseases** eg. MS, compares the electrophoretic patterns of CSF & serum immunoglobulins to find antibodies (oligoclonal bands) that are present in CSF but not in serum ie. **antibodies that are produced within the CNS**.
- **ANY intracranial inflammation** or infection will do this eg. meningitis, encephalitis, SLE & sarcoid ie. the **diagnostic power** of this test **depends on exclusion of confounding diagnoses** by clinical skills & other tests.

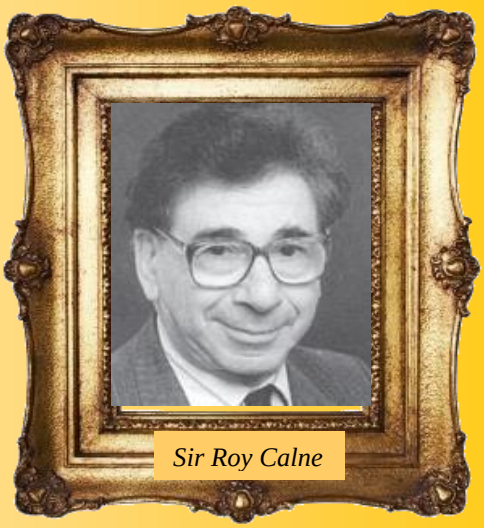
cyclosporin 3d 2mL

Sent

See sirolimus, tacrolimus, therapeutic drug monitoring & zonisamide.

- **Pre-dose sample.**
- **Repetition within 48h is rarely needed.**

Depends on use (ug/L)
Royal Sussex University Hospital & other sites if requested



Sir Roy Calne

Background

- This **calcineurin inhibitor** revolutionised transplantation by its ability to relatively safely suppress immune mediated rejection of **transplanted** organs eg. bone marrow & kidney.
- **Nephrotoxicity** is its major side effect.
- **Lower doses**, are used to treat **inflammatory diseases:**

- eczema
- psoriasis
- rheumatoid disease
- inflammatory bowel disease (IBD)

- However, **transplantation-scale doses** are used to treat **acute** worsening of IBD.

Biochemical abnormalities caused by cyclosporin

- ↑ K, creatinine, urea, uric acid, Tg, cholesterol & glucose.
- ↓ Mg
- hepatic dysfunction.

CYFRA 21-1 3d 1.5mL
See CA12.5, CA15.3, CA19.9, CEA & SCC Ag.

Sent

< 3.3 ug/L
Sheffield Protein Reference Unit

- **Do not use other ref. ranges**. Results depend on the particular antisera used in the assay.

Use

- For monitoring **squamous carcinoma** (cervix, bronchus, oesophagus, head & neck) & the response to therapy.

Background

- The protein **cytokeratin 19**, is found particularly in squamous & stratified epithelia.
- CYRFA 21-1 is the **immunoreactivity** found in plasma by an assay consisting of 2 monoclonal antibodies (BM 19-21 & KS 19-1) to epitopes in cytokeratin 19.
- The **heterogeneity** of molecular forms & antisera specificity mean that although immunoreactivity correlates with squamous cancer, the assay does not measure a clearly defined single molecule.
- In this, it is like other tumour markers eg. CA 12.5, 15.3, 19.9 & CEA.
- **Specificity is too poor for diagnosis**, but **serial results** reflect tumour progression & response to therapy.

cystine (24h urine) 3d 24h urine Sent

See amino acids (urine), C-peptide, homocystine, insulin & stone analysis.

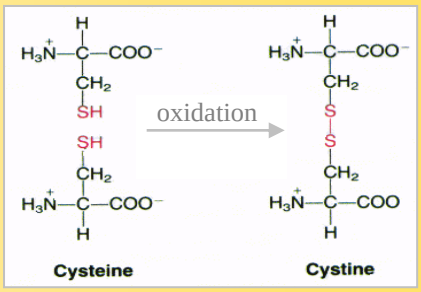
10d - 7w	18 - 28 umol/d
3m - 12y	41 - 257
Adult	< 317
Great Ormond Street Hospital	

Children only 5mL urine

0 - 1m	7 - 51 umol/mmol creat.
1 - 6m	7 - 42
6m - 1y	8 - 36
1 - 2y	6 - 28
2 - 3y	7 - 28
Great Ormond Street Hospital	

Background

- Cysteine is one of the 20 DNA encoded amino acids from which proteins are synthesised. It has an uncharged but polar sidechain ie. it can **hydrogen bond**.
- More importantly, the **sulfhydryl groups** in the sidechains of 2 cysteines can be **oxidised** & a strong covalent bond formed between their sulphur atoms – a **disulphide bond**.
- Disulphide bonds within & between protein chains are crucial to tertiary **protein structure** eg. an insulin molecule has 3. See C-peptide.
- Disulphide bonding between **2 free cysteine molecules** forms **cystine**.



Use

• Ix the 2 conditions which involve cystine:

Cystinuria – an **autosomal recessive** disease due to mutations of 2 genes, which encode components of **amino acid transporters** in the kidney & small intestine, needed for uptake from glomerular filtrate & digested food of **cystine, ornithine (a non-protein amino acid), arginine & lysine (COAL**) in the PCT. Unlike in the renal tubule, there are **alternative** transporters in the gut ie. dietary deficiences do not occur.

In the nephron, cystine is sufficiently insoluble to precipitate & form **calculi** especially under acidic or neutral conditions. However, the risk of urolithiasis is not simply related to cystine conc.

Cystine crystals are hexagonal & **stones are yellow** & can be present from the kidney to the bladder. Indeed, the cyst prefix of the amino acid name cystine refers to its first being identified nearly 200 years ago in a bladder stone. 70% of stones are pure cystine & in 30 % it is mixed with Ca oxalate, Ca phosphate or magnesium ammonium Ca phosphate. Patients mostly present in the **2nd & 3rd decades of life** with eg. UTI, pain & haematuria.

Classification of sub-types by phenotype is difficult eg. heterozygosity for 1 allele can have the same effect on cystine concentration as homozygosity for another, less disabling mutation.

Physical chemistry underlies therapy to prevent or reverse urolithiasis: 1) **drink** a lot to lower urinary cystine conc., 2) **keep urine pH up** with eg. citrus fruit juice, potassium citrate (sodium promotes crystal formation) and 3) take **drugs** which form more soluble compounds with cysteine eg. penicillamine.

Cystinosis – quite **different**. **1:200k** births. **Autosomal recessive** mutations of the gene for ***cystinosin***, a protein which normally **exports cystine from lysosomes** after production by oxidation of cysteine recycled from breakdown of proteins, causes cystine accumulation in lysosomes ie. it is a lysosomal storage disorder. Key tissues affected are:

- **Kidney** – deposition of cystine crystals in the PCT epithelium causes urinary loss of K, Na, Ca, Mg, PO_4, amino acids, bicarbonate & other molecules (**Fanconi syndrome**). Rickets, stunted growth & CRF may result.
- **Cornea** – cystine crystals causes photophobia
- **Retina** – loss of vision
- **Pancreas** – diabetes mellitus
- **Thyroid** – hypothyroidism
- **Liver**
- **Muscles** – myopathy
- **Brain** – intellectual deficit

The clinical classification of cystinosis into **nephrogenic**, **intermediate** & **non-nephrogenic** or ocular cystinosis, reflects the degree of loss of function ie. null mutations cause onset of marked widespread disease in infancy, whilst the effects of modest hypofunction may only be discovered in middle-age by an optometrist.

D

dehydroepiandrosterone sulphate (DHEAS, DHAS) 2d 1.5mL

Daily

See ACTH, androstenedione, 11-deoxycort, FAI, 17-OHP, testosterone & virilisation.

Diagram of adrenal steroid synthesis.

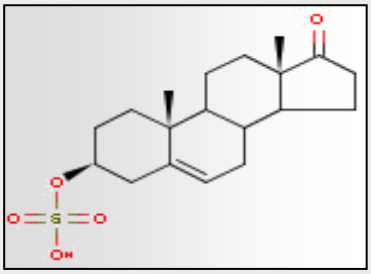
Use Ix of :

- congenital adrenal hyperplasia (CAH)
- hirsutism
- virilism
- PCOS
- amenorrhoea
- infertility
- androgen secreting tumours

Age	Female *(5^{th} - 95^{th} cent)*	Male *(5^{th} - 95^{th} cent.)*
< 1w	2.93 - 16.5 umol/L	.. umol/L
1 - 4w	0.86 - 11.7	..
1 - 12m	0.09 - 3.35	..
1 - 4y	0.01 - 0.53	..
5 - 9y	0.08 - 2.31	..
10 - 14y	0.92 - 7.60	0.66 - 6.70
15 - 19y	1.77 - 9.99	1.91 - 13.4
20 - 24y	4.02 - 11.00	5.73 - 13.4
25 - 34y	2.68 - 9.23	4.34 - 12.2
35 - 44y	1.65 - 9.15	2.41 - 11.6
45 - 54y	0.96 - 6.95	1.20 - 8.98
55 - 64y	0.51 - 5.56	1.40 - 8.01
65 - 74y	0.26 - 6.68	0.91 - 6.76
≥ 75y	0.33 - 4.18	0.44 - 3.34
Roche Cobas DHEA-S method sheet V17		

Background

- Synthesised in the **zona reticularis** of the adrenal cortex from dehydro-epiandrosterone (DHEA), a precursor to androstenedione which in turn is a precursor to testosterone (figure).
- Also synthesised in the liver from DHEA.
- DHEAS has a **plasma half-life of 1d** (much longer than DHEA itself), making it a more stable **marker of adrenal androgen output.**
- DHEAS, DHEA & androstenedione are **weak androgens**.
- Together they account for **50% of adult female androgen activity**, but they can be converted in the periphery eg. **adipose tissue**, to the more active androgen, **testosterone**.
- Rises with **adrenarche** to a peak at approx 30y & then gradually declines.



Causes of ↑

- CAH.
- Cushing's disease.
- Androgen secreting neoplasms of adrenal cortex & ovary.

Causes of ↓

- Adrenocortical hypofunction eg. Addison's disease.
- Aging

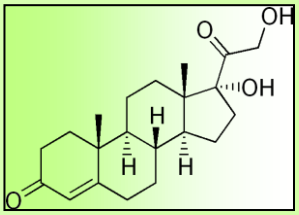
11-deoxycortisol 3d 1.5mL

Sent

< 8 nmol/L
St Helier Hospital

See androstenedione, cortisol, DHEAS, 17-OHP, renin, steroid profile & virilisation.

Use • Ix of **CAH** associated with **hypertension**.



Background

- The 2nd commonest cause of **congenital adrenal hyperplasia** (CAH) is a defect of the enzyme ***11β-hydroxylase*** (5% of cases), which catalyses the conversion of **11-deoxycortisol to cortisol** in the adrenal cortex. See DIAGRAM.
- 11β-hydroxylase deficiency causes **hypertension** (unlike most causes of CAH) because although it is active in **aldosterone synthesis**, its upregulated substrate, **11-deoxycorticosterone**, has **mineralocorticoid activity** too.

desethylamiodarone (DEA) 3d 1.5mL

Sent

0.5 – 2.0 mg/L
St Helier Hospital

See amiodarone & therapeutic drug monitoring.

Use

- **Detection of non-compliance with amiodarone therapy**.
- Recent ingestion: amiodarone/DEA concentration ratio > 3.
- Chronic therapy: levels are similar.

Background

- Amiodarone is metabolised in the liver to DEA.

digoxin 1d 1.5mL

Daily

0.9 – 2.0 ug/L
Roche Cobas Digoxin method sheet V18

See Ca, K, Mg, MRI & TDM.

- **Pre-dose sample.**
- **NOT FOR** patients on **Digibind.**
- **Check U+E** frequently if on Px which might affect serum potassium or renal function.

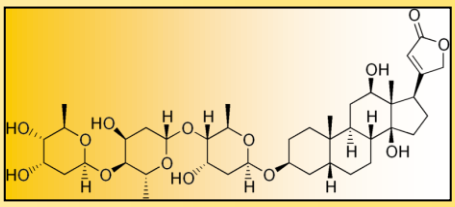
Use • optimisation of Px • Ix of toxicity

Background

- 60 - 80% of a dose is excreted unchanged by the **kidney**.
- ↓ **GFR** causes ↓ elimination & possibly toxic ↑ in serum level.
- **> 3.0 ug/L is invariably toxic** (check sample was not taken just *after* a dose).
- **Minimum retest interval** (MRI): **7 d** (5 half-lives, normal GFR) after starting or changing dose, unless toxicity suspected. If GFR ↓, allow for ↑ half-life & ↑ equilibration-time ie. ↑ MRI.
- Testing before equilibration can give **misleading results**.
- In **overdosage**, sampling may have to be as often as 4hrly depending on clinical condition & therapy.

Sensitivity to digoxin is ↑ by

- ↓ K
- ↓ Mg
- ↑ Ca
- hypothyroidism



dihydropyrimidine dehydrogenase (DYPD) 0d

Sent

See MMPN, TGN & TPMT.

- **Care with caffeine & paracetamol**. See below.

Interpretation + genotype
Purine lab., St. Thomas' Hospital

4mL 

PLUS 20mL  **urine**

Use

- Ix **risk of toxicity** from the drug **5-fluorouracil** before starting treatment.

Background

- DYPD is crucial to the elimination of the cytotoxic chemotherapy drug, **5-fluorouracil (5FU)**.
- **1 in 30** people have partially deficient activity (**carriers**) & need a smaller dose to avoid toxicity eg. half.
- **Homozygosity** causes complete absence of activity, **pyrimidinuria** & **life-threatening risk** of toxicity with 5FU doses over 5 – 10 % normal.
- **If urine screens + ve** for excess pyrimidines, the DYPD **gene is tested for common mutations** using gDNA from wbcs in EDTA blood.

Interferences

- recent blood transfusion – give date on request form.
- high caffeine intake (tea, coffee, cola, Red Bull) – abstain for 24h before urine sample.
- paracetamol – avoid .. ..

dihydrotestosterone (DHT) 1d 3mL

Sent

See 17OHP, testosterone & androgens.

Women	0.06 – 1.27 nmol/L
Men	0.86 – 3.4
Children	refs. & comment with result
St Helier Hospital	

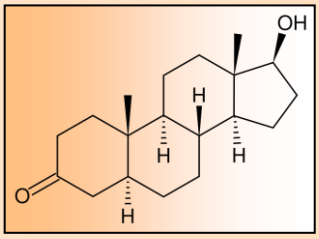
Use

- Ix of androgen resistance.

Background

- Testosterone is converted to more active dihydrotestosterone (DHT) by the cytosolic enzyme **5α-reductase** in androgen responsive tissues.
- **Deficient activity** of this enzyme can cause:

- inadequate development of male genitalia
- female phenotype in XY karyotype males if deficiency is severe.
- ↓ circulating levels of DHT, especially in response to exogenous hCG.



dopamine 3d acidified 24h urine

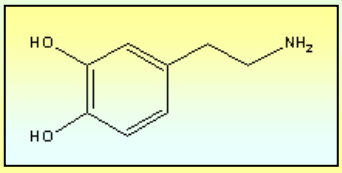
Sent

See VMA.

- **Plain bottle.**
- **Acid is added on return to the lab.**

Children only, 20mL urine 

< 3000 nmol/24h
St Helier Hospital



drugs of abuse screen 3d 20mL urine  Sent

– or + with confirmatory assay
St Helier Hospital

Background

- Only **medical** conditions related to drugs of abuse, can be investigated.
- **No recorded "chain of custody"** kept ie. results are unacceptable as evidence in court.
- Specimens **cannot be accepted in connection with occupational or legal matters**.
- **Urine is the best sample** because of the concentration of substances in it & the presence of molecules which may be in plasma only transiently. Characteristic metabolites may remain long after parent-drug-elimination.

Drug types included in screen:

- opiate
- ecstasy
- cannabinoid
- benzodiazepine
- amphetamine
- cocaine
- methadone
- barbiturate

Class-immunoreactivities are screened & + ve results are confirmed by more specific assays eg. chromatography. Other drugs can be screened. **Please ask**.

Minimum retest intervals

Amphetamine toxicity	Do not retest in the same acute episode.	ACB 2013
Benzodiazepine toxicity	ditto	ACB 2013
Cocaine toxicity	ditto	ACB 2013
Opiate toxicity eg. morphine, codeine & heroin	ditto	ACB 2013
Opioid toxicity eg. methadone	ditto	ACB 2013

Drugs & TFT results

See acute phase response, amiodarone, FT3, FT4, in vivo influences, Li, **MRI**, TDM & TSH.

Drugs				Action	typical TFT impact		
					TSH	FT4	FT3
dopamine	dopaminergic agents	glucocorticoids	octreotide	↓ TSH secretion	↓	n/↓	n/↓
lithium	iodide	amiodarone		↓ thyroid horm. secretion	↑	↓	↓
lithium	iodide	amiodarone		↑	↓	↑	↑
lithium	methimazole	carbimazole	propylthiouracil	↓ .. .. synthesis	↑	↓	↓
frusemide	NSAIDs	carbamazepine		↓ plasma protein binding	n	↓	↓
β antagonists	glucocorticoids	amiodarone	propylthiouracil	↓ T4 to T3 conversion	↑	↑	n/↓
phenytoin	barbiturates	carbamazepine	rifampicin	↑ hepatic T4 metabolism	↑	↓	n/↓
Fe sulphate	chol. binding resin	Al hydroxide	PPIs	↓ absorption from gut	↑	↓	n/↓

***NB.** Drug effects are variable & dose related, not least because they may interact with complex subclinical phenomena eg. sick-euthyroidism & compensatory physiology, which especially affects FT3 & may maintain its serum level despite changes in FT4.*

E

eGFR (estimated glomerular filtration rate) 3d See profiles

Daily

See ACR, Al, creatinine, creat clearance, K, Li, **MRI**, Na, PO_4, PSA & urea.

Use • Chronic kidney disease screening

Background

• eGFR is for screening patients at risk of **chronic kidney disease** from eg.
- diabetes
- hypertension
- heart failure
- CHD
- CVD
- PVD

(NICE CG73)

UK CKD guidelines:	
eGFR mL/min/1.73 m^2	**Comment**
> 90	Normal GFR (renal disease not excluded)
60 - 89	May be normal in the absence of structural or functional abnormality
30 - 59	Moderately impaired GFR
15 - 29	Severely .. ..
<15	Established renal impairment

• eGFR is a **better measure of GFR** than creatinine clearance or serum creatinine alone, because many people have difficulty collecting urine accurately, which degrades creatinine clearance as a measure of GFR.
• eGFR has precision errors too, but they can be easily diminished by **repetition**.
• eGFR is calculated from serum creatinine, age & gender using the **Modification of Diet in Renal Disease** (MDRD) formula. Caucasian ethnicity is assumed.
• In **Afro-Caribbean people**, multiply result by **1.21**

Do not use eGFR in:

• acute renal failure
• patients less than 18y old
• muscle wasting
• amputees
• oedematous states
• malnourished people
• pregnancy

Causes of ↓ & ↑

See serum creatinine for factors which ↑ or ↓ levels with consequent ↓ or ↑ in eGFR.

elastase 3d 5g of faeces

Sent

> 200 ug/g faeces
City Hospital, Birmingham

See amylase, calprotectin, faecal fat, **MRI**, reducing substances (faeces) & vit B12.

• **MUST have its own sample.**
• **Enzyme replacement** does not have to be stopped
- assay **measures human elastase**, not porcine enzymes as used in replacement therapy.

Use

• Ix of pancreatic exocrine insufficiency.

Background

• Elastase is a **proteolytic digestive enzyme** secreted by the exocrine pancreas.
• It is **robust** & largely survives transit to faeces where levels reflect **pancreatic exocrine sufficiency**.
• More **sensitive & specific** than faecal chymotrypsin.

Causes of ↓

• chronic pancreatitis
• cystic fibrosis
• pancreatectomy
• acute pancreatitis
• acute bacterial & viral enteritis: loss is **transient**. ? due to ↓ CCK secretion & dilution of stool by ↑ water.

ethanol 3d 2mL

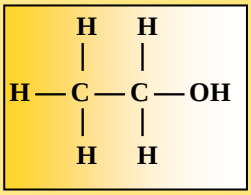
Daily

See CDT, CK, ethylene glycol, GGT, iron satn., osmo (serum), paracetamol, salicylate & TDM.

Clinical effect	Plasma level
Driving limit	800 mg/L
Flushing, blurred vision, slow reflexes	500-1000
CNS depression	> 1000
Fatalities reported	> 4000
Roche Cobas ETOH2 method sheet V10	

Use

- Ix of **medical effects** of ethanol eg. CNS depression.
- Monitoring therapy for ethylene glycol & methanol OD.



Test

- No significant interference from **methanol** or **ethylene glycol**.
- Divide mg/L by 46 to convert to mmol/L.

Background

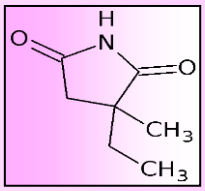
- The specimen's **"chain of custody" is not recorded** ie. the results have **no value for legal**, occupational or disputational purposes & are for **medical use only**.
- Apart from the familiar effects, ethanol excess can cause ↑CK, hypoglycaemia, ↑lactate, ↓PO_4 & ↓Mg.

ethosuximide 3d 1.5mL

Sent

See therapeutic drug monitoring.

- **Pre-dose sample.**



< 100 mg/L
St Helier Hospital

ethylene glycol 0d 2mL

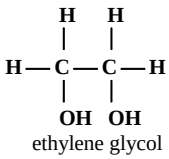
See anion gap, Ca, ethanol, lactate, osmo (serum), oxalate, paracetamol & salicylate.

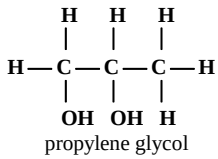
Sent

Ethylene glycol	undetectable
Diethylene glycol	..
Methanol	..
City Hospital Birmingham	

Use

- Ix ethylene glycol **poisoning.**
- osmolar gap is **non-specific screen** for glycol & alcohol ingestion.


ethylene glycol


propylene glycol

Background

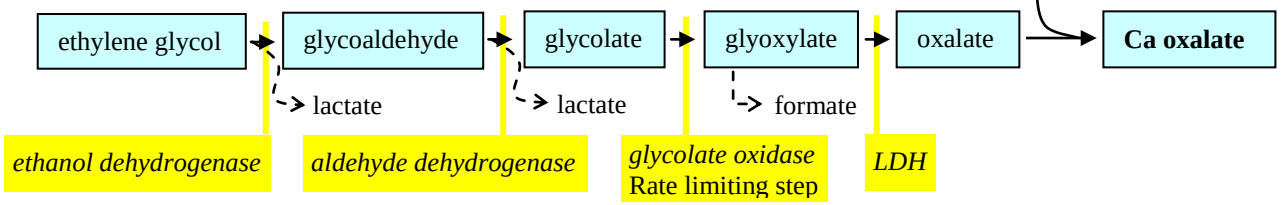
DIAGRAM of metabolism.

- Clear, **sweet** & mildly intoxicating like ethanol.
- Colour & bitter flavour are **added**.
- Found in **antifreeze**, de-icers & screen washers (often with **methanol** too), aircon. systems & cleaning fluids.
- **Propylene glycol** is non-toxic & is even added to food.
- Ethylene glycol itself is not that toxic. The problem lies in what it is metabolised to:

- **Ca oxalate**: poorly soluble & precipitates in renal tubules & parenchyma, brain, nerves, heart, vasculature, lungs
- **Sequestration of Ca** → hypocalcaemia.
- **Glycolic, glyoxylic, lactic** & **formic acid** production ↑↑ → severe high anion-gap metabolic acidosis.

- The most important is **glycolic acid** because it accumulates (conversion of glycolate to glyoxylate is rate limiting)

ethylene glycol catabolism. See oxalate DIAGRAM



- Any **methanol** present is metabolised by the same enzymes to **formaldehyde**, **formic acid** & eventually CO_2 & water. The first two metabolites are obviously highly toxic.
- Specific therapy:

- **Fomepizole** or **ethanol** (*ethanol dehydrogenase* has higher affinity than for ethylene glycol) to ↓ catabolism of glycol (& methanol). Plasma **ethanol assay** may be needed to optimise therapy.
- **Haemodialysis** to remove ethylene glycol & glycolic acid.

F

faecal fat **DISCONTINUED.**

See elastase & calprotectin.

- A **poor test in practice** (little better than clinical judgement) because of the imprecise timing of the collection.
- Faecal elastase & other tests of specific causes of steatorhoea, have replaced faecal fat assay.

faecal occult blood (FOB) See occult blood.

ferritin 3d See profiles

See acute phase, comments, CRP, folate, iron, iron satn, **MRI**, TIBC, UIBC & vit. B12.

Daily

Children	1m	150 – 450 ug/L *(5th - 95th centiles)*
	2m - 3m	80 - 500
	4m - 16y	20 - 200
Women < 60y		13 - 150
Men (all ages) + **Women** > 60y		30 – 400
Roche Cobas Ferritin method sheet V9 & Ref. Ranges for Adults & Children 2004		

Use

- A marker of iron stores.

Background

- A 24 subunit protein shell (**apoferritin**) with a core of 2500 **ferric** (Fe^{3+}) ions per molecule, on average.
- **Isoforms** exist, which differ in the number of protein subunits.
- Approx. **1g of iron** is stored **in ferritin** & **haemosiderin** (aggregated ferritin) in the reticuloendothelial system, liver, spleen & bone marrow.
- Approx. **2.5g of iron in Hb**, where it is Fe^{2+} (ferrous).
- Serum ferritin is an **indicator of iron stores** because it is in equilibrium with storage ferritin. Contrast this with serum iron, which is only 0.1% of total body content & an unreliable index. Iron saturation is better but changes little until functional iron stores are exhausted.
- **NB**. serum ferritin levels **may be normal despite iron deficiency** & **may be ↑ when iron stores are not**, because of influences in addition to iron, especially inflammation. See below.
- **Double check abnormal ferritin** results with serum iron saturation.
- Interpret serum ferritin cautiously if **CRP** is ↑.

Causes of ↑

- Fasting / wasting
- ↑ Age
- ↑ iron stores eg. haemochromatosis, aplastic anaemia, thalasaemia, CRF
- Inflammation eg. Rh, SLE, infection & surgery ie. **ferritin is a + ve acute phase reactant like CRP**.
- Acute & chronic liver disease eg. alcoholic & inflammatory hepatitis.
- Chronic haemolysis
- Malignant diseases eg.
  - leukaemia
  - lymphoma
  - carcinoma of lung, breast, colon, prostate & liver
- For a few days after parenteral iron.

Causes of ↓

- iron deficiency.

flecainide 3d 3mL

See therapeutic drug monitoring.

- **Pre-dose sample.**

Sent

200 – 700 ug/L
Llandough Hospital

Use

- Optimisation of dose.

Background

- Prevention & treatment of **tachyarrhythmias** eg. paroxysmal AF & SVT.
- Most of a dose is excreted in **urine**, but some is metabolised by hepatic CYP2D6.
- The narrow therapeutic index, sensitivity to ↓ GFR & effect of other drugs, make **monitoring useful**.

Causes of ↑

- ↓ GFR
- Inhibition of CYP2D6 by amiodarone & cimetidine.

Biochemical effects of flecainide

- ↑ digoxin levels

folate (RBC) 1d 4mL

See folate (serum), vit. B12, homocystine & methyl malonic acid.

DISCONTINUED. Replaced. See below.

Use

- Ix of folate deficiency if serum folate results are inconclusive.

Background

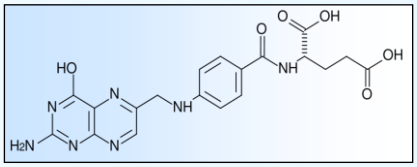
- Said to reflect adequacy of folate levels at the intracellular site of action more accurately than serum folate does.
- However, this advantage is **degraded** by the multiple variances involved in calculation of rbc folate.
- Rbc folate assay has been **replaced by plasma homocystine**, a marker of ↓ folate & vit. B12 function.

folate (serum) 2d see profiles

Daily

See ferritin, folate (rbc), homocystine, iron, iron satn, methylmalonic acid, **MRI**, TIBC, UIBC, vit. B6, vit B12 & Zn.

4.6 – 18.7 ug/L *(2.5th – 97.5th centile)*
Roche Cobas Folate III method sheet V11



Use

- Ix of deficiency.

Background

- **Source**: green leafy veg., brown rice, fruit, supplemented bread & cereals.
- Absorbed in **jejunum**
- Risk of **neural tube defects** in babies of mothers, even on an apparently good diet, is less if a **supplement** of 0.4 mg/day is taken from pre-conception to 12/40 gestation. Women at ↑ risk (PMH or FH of NTD, diabetes, BMI > 30 & some drugs eg. anticonvulsants), should take a dose of up to 5mg/day. Do not supplement vit. A.
- Folate is usually **assayed with B12** since megaloblastic anaemia can be due to lack of either & most importantly, replacing folate alone can precipitate neuropathy if vit. B12 deficiency is also present.
- Folate, like vit. B12, has a key role in synthesis of intermediary metabolites, especially for methylation reactions eg. for DNA & RNA synthesis. See homocystine DIAGRAM.

Causes of ↓

- **deficient diet**
  - alcoholism
  - drug addiction
  - self-neglect
  - urban poverty
  - heating & boiling destroys folate
- **malabsorption**
  - sprue
  - coeliac disease
  - bowel resection
  - IBD
- **↑ demand**
  - pregnancy
  - severe dermatitis

free androgen index 2d 1.5m

Daily

See DHEAS, SHBG & testosterone.

Women	20 – 49 y	0.30 – 5.62 %
	≥ 50	0.19 – 3.63
Men	20 – 49 y	35.0 – 92.6
	≥ 50	24.3 – 72.1
Roche Cobas SHBG method sheet V11 & Testosterone II method sheet V7 (5th - 95th cent.)		

Use

- Ix significance of **total testosterone** results.

Background

- The biological significance in women of serum total testosterone in the upper half of the ref. range ie. > 1.0 nmol/L, relates to the level of sex hormone binding globulin (SHBG) because **bound hormone is "inactive"**.
- **"Unbound"** hormone is actually a mixture of **free** & **albumin-bound** testosterone. Binding to albumin is too weak to affect bioavailability. This "bioactive" testosterone is **estimated** by the Free Androgen Index (FAI):

FAI = testosterone conc. (nmol/L) x 100 ÷ SHBG conc. (nmol/L)

FEMALES:

- SHBG assay is **automatically added** to testosterone results **> 1.0 nmol/L**.
- Total testosterone levels in the ref. range can be physiologically significant if SHBG is ↓.

MALES:

- SHBG assay is **automatically added** to testosterone results **< 14.0 nmol/L** & above the upper ref. limit.
- It is not uncommon for "low" serum total testosterone with "normal" FAI to be due to variant "low" SHBG.

free fatty acids 0d 1.5mL Sent

See β hydroxybutyrate, carnitine, C-pep., glucose, glycolipid, insulin & Tg.

0.28 – 0.89 mmol/L

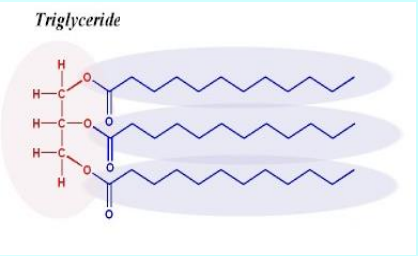
Great Ormond Street Hospital

Use

- Ix of **hypoglycaemia** – insulin causes ↓ lipolysis, ↓ serum FFA & ↓ ketone body synthesis (β hydroxybutyrate).
- Ix of **fatty acid oxidation defects** – FFA levels ↑ + there can be abnormalities of carnitine & β hydroxybutyrate.

Background

- Free means **non-esterified** ie. carboxylic acids without ester linkage as in triglyceride & phospholipids, rather than not protein-bound eg. Ca.
- Circulating "FFA" is **actually highly protein bound** eg. to albumin.



Free fatty acid

free light chains (FLC) serum 3d 1.5 mL

Sent

free **kappa** chains	3.3 – 19.4 mg/L
free **lambda** ..	5.7 – 26.3
K/L **ratio**	0.26 – 1.65
Sheffield Protein Reference Unit	

See β_2M, BJP, cryoglobulins, FLC (urine), IgG subclasses, Igs & serum protein electro.

- **For the Haematology Team only, please.**

Use

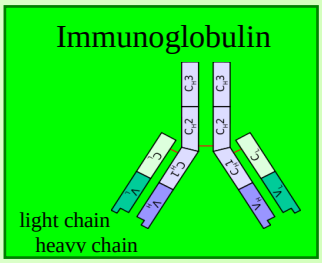
- Monitoring **BJP** & **"non-secretory" myeloma**.
- Monitoring paraproteins **vulnerable to artefact** eg. cryoglobulinaemia, Waldenstrom's macroglobulinaemia.
- Ix & monitoring of **MGUS** (monoclonal gammopathy of undetermined significance).
- Increasingly, FLC assay is added to standard methods eg. BJP to ↑ **sensitivity** & for **prognostication**.

Background

- Intact Ig molecules are made of **sulphur-bonded pairs of heavy & light chains** (see below).
- Free (not bound to a heavy chain) light chains (MWt **23 kDa**, *cf.* albumin 66kDa) readily enter glomerular filtrate.
- In urine, FLCs are termed **Bence Jones Protein**.
- Plasma levels ↑ with **neoplastic** & **benign ↑ Ig synthesis** eg. chronic infection & connective tissue disorders.
- FLC assays **cannot distinguish polyclonal from monoclonal ↑** due to inflammation & B cell malignancy, respectively, although an abnormal K/L ratio can result from the less ordered synthesis in neoplasia.
- Only **immunofixation** (electrophoresis & staining with antisera to specific heavy & light chains) can identify monoclonality & confirm the nature of FLC.
- In **non-secretory myeloma** though there is no paraprotein or BJP, FLC may still be detectable in serum & can be used to monitor tumour bulk & response to therapy.

Cause of ↑

- myeloma
- ↓ GFR
- B-cell CLL
- Waldenstroms macroglobulinaemia
- plasmacytoma
- B-cell NHL
- plasma cell leukaemia
- infection
- cryoglobulinaemia
- connective tissue disorders
- POEMS syndrome



free light chains (FLC) urine 0d 20 mL Early Morning Urine

Sent

Ref. range sent with result
Sheffield Protein Reference Unit

See FLC serum, BJP, BJP quantification & serum protein electrophoresis.

- **For the Haematology Team only, please.**

Use

- **Serum FLC** is more sensitive, precise & practicable than urinary FLC excretion.
- Urine testing may suit patients who loath venepuncture.

Background

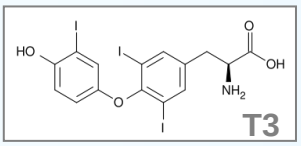
- See FLC (serum)
- FLCs **readily enter glomerular filtrate** because of their small size – 23kDa.
- **Reuptake** occurs in the PCT, which **↓ the sensitivity** of urinary FLC as a marker of ↑ production.
- A **24h urine** specimen improves performance but it is **inconvenient** & **inaccurate** in practice.
- A combination of serum FLC assay with urine protein electrophoresis (BJP assay) has higher performance for detection of B cell neoplasia eg. myeloma, than urine FLC.

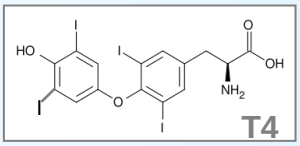
free T3 (tri-iodothyronine) 3d See profiles

Daily

See drugs & TFTs, FT4, **MRI**, thyroglobulin, TRH test & TSH.

- **Give clinical details** eg. on T4, on T3, pregnant…





Children	0 - 3d	3.0 - 12.1 pmol/L
	4d - day before 2m old	3.0 - 8.0
	2m - day before 2nd b'day	2.4 - 9.8
	2y - day before 7th b'day	3.0 - 9.1
	7y - day before 12th b'day	4.1 - 7.9
	12y - 19th b'day	3.5 - 7.7
Adults	women	3.7 - 6.7
	men	4.1 - 6.6
Pregnancy	1st trimester	3.8 - 6.0
	2nd	3.2 - 5.4
	3rd	3.1 - 5.0

Roche Cobas FT3 method sheet V15. Ref. Intervals for children & adults. Elecsys Thyroid Tests 2004. (2.5th - 97.5th centiles)

Use

- Ix of normal FT4 with a TSH ≤ 0.1 mU/L
- Monitoring T3 Px.
- Ix ambiguous TSH & FT4 results eg. on amiodarone.
- To confirm ↑ FT4 at diagnosis of hyperthyroidism.
- Ix assay interference.
- Ix thyroid hormone resistance.
- Ix of sick-euthyroidism & 2ndy hypothyroidism

See ***"The UK Guidelines for the Use of Thyroid Function Tests"*** for evidence-based tips on the use & interpretation of TFTs in a wide range of conditions. Available on the www & SASH intranet.

Background

- **TESTING MORE OFTEN THAN MONTHLY IS UNNECESSARY** & can give misleading results, because it takes at least this long for serum FT4 levels to equilibrate. T4 plasma **half-life = 1 week**.
- **Thyroxine** (T4) is a relatively **inactive precursor** (prohormone) **to triiodothyronine** (T3), the active hormone.
- Only **0.5 %** of total plasma T3 is not protein bound (free & bioactive).
- 20 % of T3 is secreted directly by thyroid.
- 80 % is from **deiodination of the outer ring of T4**, principally in the liver & kidneys, but also in target tissues.
- **Conversion** of T4 → T3 is ↓ by:

- systemic illness
- prolonged fasting
- drugs eg. amiodarone & β blockers.

- T3 is **inactivated** by deiodination of the inner ring, creating **diiodothyronine** (T2). Deiodination of T4 at this site creates the inactive compound, **reverse T3** (rT3).
- FT3 is subject to **non-thyroidal influences** & changes much less with thyroid abnormality than TSH does ie. FT3 is a **poor marker of thyroid function**, except in restricted circumstances.
- Despite hope that FT3 would gauge **T4 replacement**, it has **NOT** proved to be **reliable** eg. in primary hypothyroidism, FT3 may ↓ as the T4 dose ↑, perhaps due to ↓ T3 secretion by thyroid as TSH ↓ with replacement.
- **Interpret FT3 cautiously** if on T3 or combined T4 + T3 replacement: the more **dynamic pharmacokinetics** of T3 vs. T4, make FT3 results vulnerable to artefacts eg. ↑ due to blood sampling in the distribution phase.

free T4 (thyroxine) 2d See profiles

Daily

See drugs & TFTs, FT3, Li, **MRI**, thyroglobulin, TRH test & TSH.

- **Give clinical details** eg. on T4, T3, pregnant…

Children	0 - 3d	8.5 – 34.9 pmol/L
	4d - day before 2m old	10.6 – 39.8
	2m - day before 2nd b'day	6.2 – 30.1
	2y - day before 7th b'day	11.0 – 22.5
	7y - day before 12th b'day	11.6 – 21.5
	12y - 19th b'day	12.0 – 20.6
Adults	Women	12.3 – 20.2
	Men	13.1 – 21.3
Pregnancy	1st trimester	12.0 – 19.6
	2nd	9.6 – 17.0
	3rd	8.4 – 15.6
Roche Cobas FT4 method sheeet V19 & Ref. Intervals for Children & Adults. Elecsys Thyroid Tests. 2004 (2.5th – 97.5th centiles).		

Use

- **TSH** is the single most-useful TFT.
- Addition of **FT4 is essential**:
- if **TSH response is** ↓ eg. hypopituitarism & thyrotrope atrophy due to hyperthyroidism or XS replacement. See TSH.
- in **pregnancy** – maternal FT4 correlates with foetal well-being better than TSH.
- FT4 assay is **automatically added** if TSH result is outside ref. range.

See ***"The UK Guidelines for the Use of Thyroid Function Tests"*** for evidence-based tips on the use & interpretation of TFTs in a wide range of conditions. Available on the www & SASH intranet

Background

- **Testing more often than monthly is unnecessary** & can give misleading results, because it takes at least this long for serum FT4 levels to equilibrate. T4 plasma **half-life = 1 week**.
- Thyroid hormones regulate growth, development, energy & protein, lipid & carbohydrate metabolism.
- **Iodide** (I^-) is actively taken up by thyroid follicular cells & converted to **iodine** by **peroxidase** (blocked by carbimazole & propylthiouracil) for iodination of **tyrosine** residues in the protein **thyroglobulin** to form mono & diiodotyrosines. Coupling of these forms T3 & T4. See FT3 diagram.
- Plasma T3 & T4 feedback to inhibit TRH & TSH secretion, which results in ↓ thyroid hormone secretion.
- T4 is less bioactive than T3 & is actually a **prohormone** which must first be converted to T3 by **5′ deiodinase** in the target cell cytoplasm in order to bind its receptor.
- **Only 0.05 % of plasma total T4 is not protein bound** ie. free & bioavailable. FT4 assays are much less affected by protein binding changes than total hormone ones. But they are not immune eg. congenital variants of thyroxine binding globulin & albumin can distort FT4 assays to give misleading results eg. ↑ FT4 but normal TSH.
- The **signal path from plasma to nuclear receptors** has many steps, all open to pathophysiological influence eg.
- transport of T4 across the cell membrane via **L-amino acid transporters**, **organic anion transport proteins** (OATPs) & **monocarboxylate transporters** (MCT8, MCT10).
- conversion of T4 to T3 by cytoplasmic **5′ deiodinase.**
- T3 binding to cytoplasmic **monomeric receptors** which then cross the nuclear membrane to form homo & hetero**dimeric transcription factors**.
- All these proteins have subtypes with tissue specific distribution!
- **Results must always be interpreted in their clinical context**, because of this complexity.

T4 replacement.

- In **primary hypothyroidism**, **TSH** is the principal guide when it is not suppressed. The **FT4 ref.** range is based on normals with **endogenous** thyroid hormone production. FT4 **levels are higher in patients on T4** for primary hypothyroidism eg. the upper ref. limit has been reported as 26 pmol/L in clinically euthyroid patients on T4. This relates to ↓ secretion of T3 by the thyroid in response to the ↓ TSH following replacement. See FT3.
- **Titrate the dose against TSH**, not FT4, except in **pregnancy, hypopituitarism, thyrotrope atrophy** etc.

Sick-euthyroidism. See acute phase response.

- In illness eg. surgery, MI & inflammation, the energy & substrate-economy needed for recovery is different from the well-state. TFT results change as part of this process:
- TSH: ↑ within 24h of onset of acute illness, but is later normal or ↓
- FT4: N or ↓ (sometimes transiently ↑ too, especially when recovering).
- FT3: N or ↓
- The typical picture is often not seen & results are just distorted ie. **do not request TFTs unless indicated.**

fructosamine 3d 1.5mL

See HbA1c & fasting glucose.

+ ve interference: levodopa
oxytetracycline

Twice a week.

No diabetes	205 - 285 umol/L
Good control	264 - 320
Poor ..	228 - 563
Roche Cobas FRA method sheet V6 & Tietz	

Use

- **A substitute for HbA1c** for monitoring diabetes in patients with ↓ rbc lifespan or with haemoglobinopathy.

Background

- Produced by **non-enzymatic glucose glycation of plasma proteins, mostly albumin**.
- Like HbA1c, fructosamine conc. provides a **time-averaged indicator** of plasma glucose levels.
- The 19d half-life of albumin means that fructosamine reflects glucose control over **1 – 2 months** compared with **2 – 3 months with HbA1c**.
- The shorter time is not as useful as the independence of Hb ie. fructosamine is an **alternative to HbA1c** when:

- HbA1c is ↓ by ↑ rbc turnover eg. due to **haemolysis**, which can hide poor glycaemic control.
- abnormal Hb forms are present (**haemoglobinopathy**) which can misleadingly ↑ or ↓ the HbA1c result.

Causes of ↑

- hyperglycaemia
- L-DOPA

Causes of ↓

- hypoalbuminaemia (<30g/L)

FSH (Follicle Stimulating Hormone) 2d See profiles

See AMH, hCG, inhibin B, LH, LHRH test, **MRI**, oestradiol, prolactin, progesterone & testosterone.

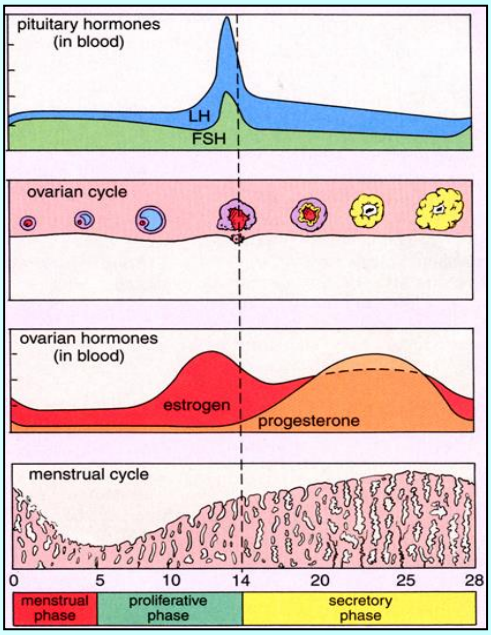
Daily

Background

- A **heterodimeric** glycoprotein made of a
- 92aa **α-chain** (the same as in TSH, LH & hCG) with a
- 118aa **β-chain** (sets the unique FSH properties).
- Secreted by pituitary **gonadotropes** in response to hypothalamic gonadotropin releasing hormone (GnRH or **LHRH**).

Women	Follicular phase	3.5 - 12.5 IU/L
	Mid-cycle peak	4.7 - 21.5
	Luteal phase	1.7 - 7.7
	Postmenopause	25.8 - 134.8
Men	20 - 60y	1.5 - 12.4
Roche Cobas FSH method sheet V19 (5th - 95th centiles)		

- **Secretion is inhibited by inhibin B** secreted by testicular **Sertoli** & ovarian **granulosa cells** of mature follicles, as a result of FSH action ie. – ve feedback.
- **Sex steroids** have a role too, especially in women, where oestradiol contributes to transient +ve feedback & midcycle LH & FSH peaks



MEN

- FSH regulates:
- **Sertoli cell growth**
- **spermatogenesis**
- **seminiferous tubule growth**.

WOMEN

- FSH effects in women are more complex & relate to the menstrual phase. Broadly, FSH stimulates:
- **follicle recruitment & growth** in the follicular phase. Inhibin, secreted by growing follicles, feeds back & inhibits FSH secretion.
- **oestradiol synthesis**, especially in the follicular phase.

Causes of ↑ Primary hypogonadism eg.

- Kleinfelter's syndrome (males)
- Turner's syndrome (females)
- **Menopause**, ↑ serum FSH is the most sensitive indicator, but in the time between full reproductive activity & the menopause (**perimenopause**), FSH & oestradiol levels can be very variable & at times appear to be almost independent of each other. Ix by repeating FSH test in 1 – 2 w.
- orchitis, trauma etc.

Causes of ↓ Secondary hypogonadism (hypogonadotropic hypogonadism) eg.

- pre-puberty
- physiological response eg. ↓ Wt., debilitation
- LHRH analogues
- exogenous sex steroids
- Kallmann's syndrome
- hypopituitarism
- hypothalamic disease
- pregnancy (not hypogonadism, but placenta controls ovaries & -ve feedback turns off pituitary FSH & LH)

G

gabapentin 3d 1.5mL

Sent

≤ 24 mg/L
St Helier Hospital

See pregabalin & therapeutic drug monitoring.

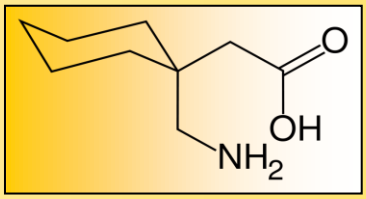
- **Pre-dose sample.**

Use

- For Ix of **toxicity**, **compliance** & when **control is difficult** to establish or is inexplicably lost.

Background

- For treating epilepsy, neuropathic pain & bipolar disorder.
- Most is **excreted unchanged** in urine.
- Originally synthesised to mimic the inhibitory neurotransmitter GABA (**γ aminobutyric acid)**, but its action involves additional mechanisms.



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galactose-1-phosphate uridyl transferase 0d 2mL

(Gal-1-PUT) See reducing substances (urine), sugar chromatography & wbc enzymes.

Sent

- **MUST** be in ESH lab. by **10am.**
- **MUST** get to GOSH by **2pm.**
- **DO NOT** send on **Friday**.
- **Invalidated** by blood transfusion in last 6w.

Normal	18 - 40 umol/h/g Hb
Galactosaemia homozygous	0.0 - 6.0
.. .. heterozygous	9.0 - 15.0
Great Ormond Street Hospital	

Use Ix of galactosaemia.

- **Measurement of the enzyme is preferred** to examination of urinary **reducing subsances** as a screen for galactosaemia, because **false negatives** can occur, especially if lactose is not in the diet

Background

- Dietary **lactose** is hydrolysed to **glucose** & **galactose** by lactase in intestinal mucosa.
- The liver converts **galactose** to **glucose in 3 steps** involving **3 cytoplasmic enzymes** which can be congenitally deficient**:**

- galactose-1-phosphate uridyl transferase (Gal-1-PUT): **classical galactosaemia**
- galactokinase
- UDP galactose 4-epimerase.

- **Classical** galactosaemia (1:70,000) is a recessively inherited deficiency of **GAL-1-PUT** with clinical presentation ranging from severe **unconjugated jaundice** with liver failure to a chronic disorder with cataracts, liver disease & learning difficulties. Galactose, galactitol & galactose 1-phosphate accumulate in all tissues & *may* give +ve results for **reducing substances in urine** (see above).
- Rarely, galactosaemia is due to defects of the other 2 enzymes.

.

α-galactosidase A 0d 3mL

(*ceramide trihexosidase*, **Fabry's disease**).
See GAGs, glycolipid & wbc enzymes.

Sent

Refs sent with results.
Great Ormond Street Hospital

- **MUST** be in ESH lab. by **10am.**
- **MUST** get to GOSH by **2pm.**
- **DO NOT** send on **Friday**.
- **Invalidated** by blood transfusion in previous 6w.

Use Ix & diagnosis of **Fabry's** disease.

Background

- **Fabry's** disease a **lipid storage dis.** (lipidosis) is due to deficiency of the **lysosomal** enzyme α-galactosidase A.
- **X-linked**, 1:50k males. **Carriers** can have clinical features if lyonisation reduces the normal allele sufficiently.
- **Variant alleles** causing only partial loss of enzyme activity **may present in adulthood** as isolated renal or cardiac disease eg. unexplained LVF.
- **Onset** – usually in children & adolescents eg. painful peripheral neuropathy • dermopathy (angiokeratomas) • ↓ sweating • corneal opacities • coronary & cerebro arterial disease (MI & stroke) • cardiac enlargement • CCF • CKD • gastrointestinal dysfunction (autonomic neuropathy).
- **Globotriaosylceramide** (Gb3) & **galactosylceramide** (glycosphingolipids) accumulate in lysosomes because catabolism cannot go further if terminal galactose (galactosyl) residues are not removed by α-galactosidase A.
- Deficiencies of **other lysosomal enzymes** which dismantle the carbohydrate portion of glycosphingolipids, also cause lipidoses & related clinical phenomena eg. Gaucher's, Krabbe's, metachromatic leukodystrophy & Tay Sachs diseases.
- **Recombinant** galactosidase can be given by ivi. It is taken up by **mannose-6-phosphate receptors** on the cell surface & directed to the lysosomes, especially in vascular endothelium, with ↓ of the lipid inclusions.

gamma-glutamyl transferase 3d See profiles
See ALP, ALP isozymes, ALT, AST, bilirubin, CDT, CK, comments, ethanol, iron satn & **MRI**.

Daily

Women	6 – 42 U/L
Men	10 – 71
Roche Cobas GGT-2 IFCC method sheet V5	

Uses

- Detecting & monitoring **ethanol abuse** (induces hepatic GGT). **BUT….** See below
- **Ix cause of ↑ ALP**. GGT & hepatic ALP are both induced by cholestasis of *any* cause

Background

- **Present in all tissues** (except muscle) ie. not just a liver-enzyme.
- Highest levels in **liver, kidney** & **pancreas** where most is located in membranes, including the cell membrane.
- GGT has a role in **transport** of amino acids & peptides into & out of cells.
- **Specificity** & **sensitivity for ↑ ethanol intake are poor** eg.

- also induced by eg. **phenytoin, phenobarbitone, pancreatitis** & **cholestasis** of *any* cause.
- serum GGT is *normal* in approx. half of alcoholics. If ↑, it can fall to normal after 6 - 8 w abstinence.
- GGT level & quantity of ethanol consumed correlate poorly.

Causes of ↑

- Cholestasis of any cause eg. cirrhosis, hepatitis, liver metastases, stones, drug effect.
- Induction by drugs eg. phenytoin & phenobarbitone.
- .. .. chronic ethanol excess.
- Pancreatitis
- Hyperthyroidism

Causes of ↓

- Hypothyroidism

gastrin 0d 3mL

Sent

See gut hormone profile.

0 – 40 pmol/L
Charing Cross Hospital

- **Keep on ice.**
- **No aprotinin needed** for gastrin alone. - **BUT** needed if part of a gut hormone profile.
- **Fasting** essential (10 – 16h). - Food ↑ plasma gastrin.
- **Off PPIs** for 14d & **H2 blockers** for 3d. - These drugs ↑ plasma gastrin.
- **Clinical details** needed

Background

- Secreted by **G cells of gastric antrum**, gastrin stimulates HCl secretion by parietal cells in the gastric mucosa.
- In the **Zollinger - Ellison syndrome**, hypersecretion of gastrin by a neuroendocrine tumour in the pancreatic islets (25%) or duodenum (70%), causes recurrent severe peptic ulceration, diarrhoea & steatorrhoea (acid inhibition of pancreatic lipase). 7% of cases have diarrhoea without ulcers. 60% of gastrinomas are malignant & 50% have metastasised at diagnosis. 70% are sporadic, but 25% are part of Multiple Endocrine Neoplasia type 1 (**MEN1**) (primary hyperparathyroidism, entero-pancreatic tumours, anterior pituitary adenomas).
- A gut hormone profile may be more suitable than gastrin alone if **MEN1** is a possibility, because there may be additional tumours eg. glucagonomas & VIPomas (vasoactive intestinal polypeptide).

Causes of ↑

- **not fasting**
- achlorhydria
- renal failure
- **drugs** – PPIs, H2Bs
- antral G-cell hyperplasia
- hypercalcaemia, .
- partial gastrectomy
- vagotomy

gentamicin 3d 1.5mL

Daily

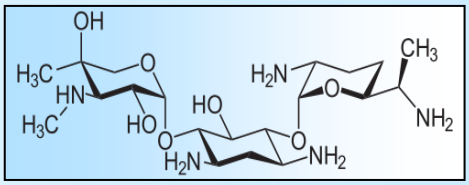
See amikacin, comments, TDM, tobramycin & vancomycin.

mg/L
Roche Cobas GENT2 method sheet V2

Contact Microbiology for advice on interpretation & patient management.
See IPCAS on the SASH intranet for sample timing, target levels, responses to results, dose etc.

Use

- For achieving an **effective dose with minimal toxicity**, especially if there is:
- serious infection crucially requiring effective levels
- ↓ or changing GFR
- inadequate therapeutic response
- variant pharmacokinetics eg. in obese, febrile or neonatal patients
- evidence of ototoxicity.



Background

- > 90% **eliminated in urine**, thus plasma levels ↑ with ↓ GFR.
- Plasma **half-life is 2 – 3 h**, but can be nearly 100h in renal failure.
- **Nephro** & **ototoxic** like other aminoglycoside antibiotics, but ineffectively small doses only promote resistance. This balance is especially important in the elderly, patients with ↓ GFR & those with cystic fibrosis.

glucagon 0d 6mL + **aprotinin** Contact lab.

See glucagon stim. test, glucose & gut hormone profile.

- **MUST be fasting**
- **Keep on ice.**
- **Get to lab. asap.**

Sent

Adults, normal	< 50 pmol/L
Typical glucagonoma	> 200
Charing Cross Hospital	

Use

- Ix of suspected **neuroendocrine tumours** as part of a gut hormone profile.
- Ix of glucagon **hypersecretion**, which has syndromic features. See below.
- Ix of glucagon **deficiency**: a rare +++ cause of hypoglycaemia in babies.
- In the glucagon stimulation test, in which glucagon is given to stimulate pituitary secretion of GH & ACTH, **glucagon itself does not need to be assayed.**

Background

- Glucagon (29aa) is a major insulin counter-regulatory hormone secreted by the **islet A (α) cells**.
- It increases plasma glucose principally by promoting **glycogenolysis** & **gluconeogenesis** in the liver.
- In stress, starvation & other states where insulin action is low eg. diabetes mellitus, glucagon stimulates **lipolysis**, FFA export & **ketogenesis**.
- All **glucagonomas** are pancreatic, cause diabetes, fat & muscle catabolism, Wt. loss, venous thrombosis & a characteristic skin lesion – **necrolytic migratory erythema**. 75% are malignant & 75% have metastasised at diagnosis, but unlike carcinoids, **liver mets. are unnecessary for syndromic clinical features** to be seen. Rarely, glucagonomas are part of the MEN1 syndrome (FH of pituitary, enteropancreatic & parathyroid adenomas).

glucose (CSF) 3d 0.2mL

See CSF oligoclonal bands, CSF protein, glucose (random) & xanthochromia.

- **Measure plasma glucose too**, to improve interpretation.

Daily

Children	3.3 - 4.4 mmol/L
Adults	2.2 - 3.9
both	> 60 % of plasma glucose
Roche Cobas GLUC3 method sheet V4	

Use

- Ix meningitis & encephalitis.

Causes of ↓

- viral meningitis
- SAH

Causes of ↓↓

- bacterial/TB/cryptococcal meningitis
- mumps encephalitis
- 1y or 2ndy neoplasia of meninges
- sarcoidosis

glucose (fasting, venous plasma) (FPG) 3d 1mL

Daily

See ACR, β-hydroxybutyrate, carnitine, comments, C-peptide, FFA, fructosamine, GH suppr test, glucagon, glucagon stim. test, glucose (CSF), glucose (random), HbA1c, insulin & OGTT.

Use

- Ix & screening for DM & glucose intolerance.
- Ix hypoglycaemia.

Normal	3.0 - 6.0 mmol/L
Impaired Fasting Glucose	6.1 – 6.9
Diabetes mellitus	≥ 7.0
Roche Cobas GLUC3 method sheet V4 & WHO 2006	

Comments

- If in doubt, use an oral glucose tolerance test (OGTT).
- **In the absence of symptoms**, diagnosis of DM should not be based on 1 abnormal result.
- **N.B.** The American Diabetes Association (ADA) suggests **a lower fasting plasma glucose for definition of IFG** than the WHO does ie. 5.6 – 6.9 rather than 6.1 – 6.9 mmol/L. See ADA 2011 & WHO 2006.

glucose (random, venous plasma) 3d 1mL

Daily

See β-hydroxybutyrate, carnitine, comments, FFA, glucose (CSF), glucose (fasting), HbA1c & OGTT.

Use

- Ix of DM & hypoglycaemia at the time of symptoms.
- To accompany assay of CSF glucose.

≤ 6.0 mmol/L	**Normal**
6.1 – 11.0	**Repeat** while fasting
≥ 11.1 + symptoms	**DM**. If unclear, repeat fasting
Roche Cobas GLUC3 method sheet V4 & WHO 2006	

In the absence of symptoms, diagnosis of DM should not be based on just 1 abnormal result.

glucose tolerance test (Oral GTT) 3d 1mL

Daily

See GH suppr. test, glucose fasting, glucose random & HbA1c.

Use Ix

- DM
- IFG
- acromegaly
- reactive hypoglycaemia

Fasting plasma gluc.	2h post 75g gluc.	Interpretation
≤ 6.0 mmol/L	< 7.8 mmol/L	Normal
6.1 – 6.9	< 7.8	IFG - impaired fasting glucose
< 7.0 & ……..	7.8 – 11.0	IGT - impaired glucose tolerance
> 7.0 or ……..	≥ 11.1	DM - diabetes mellitus
Roche Cobas GLUC3 method sheet V4. WHO 2006		

- **The American Diabetes Association suggests a lower IFG limit** than WHO ie. 5.6 – 6.9 vs. 6.1 – 6.9 mmol/L. See ADA 2011 & WHO 2006.

Ix of DM, IGT & acromegaly

PROCEDURE

- Only needed if diagnosis is equivocal eg. there is IFG.
- **There must be:**
  - normal feeding in the previous 3 d
  - an overnight fast
  - no snacks, no breakfast, no tea, no coffee. Can have H_2O.
  - no stress from illness or surgery.
  - **75g anhydrous glucose** in 200 - 300mL water, (Alternatively, 113 mL of **Polycal** diluted to 200 - 300 mL)
  - drink in 3 – 5 min. without nausea (delays gastric emptying)
  - **DO NOT use Lucozade** (see GDM screening below).
  - resting.
  - no smoking during OGTT.
- For Ix of **diabetes**, take fluoridated venous blood (grey tubes) at 0 & 120 min.
- .. .. .. **GH secretion**, samples for glucose & GH at 0, 30, 60, 90 & 120 min.
- .. .. .. **reactive hypoglycaemia**, samples for glucose at 0, 30, 60, 90, 120, 150, 180, 210 & 240 min.

INTERPRETATION See references above

- Patients with IFG, IGT & even those classified as "normal" by the OGTT, should ↓ CVD & DM-risk factors (↑ cholesterol, smoking, ↑ BP, obesity & ↓ exercise) & test fasting plasma glucose eg. annually. See HbA1c.
- Ix acromegaly: see GH suppression test.

Screening for gestational diabetes mellitus (GDM) – simplified GTT

PROCEDURE & preparation as above, except:

- 75g of anhydrous glucose is nauseating. **Lucozade** (a solution of glucose & glucose-oligomers from incompletely hydrolysed starch) is more pleasant & the oligomers are rapidly digested to monomeric glucose.
- **410 mL of *Lucozade*** *Energy Original* (**70 kcal/100 mL**) = 75g of anhydrous glucose.
- **Take blood** for plasma glucose (grey cap) just before (optional) & 120 minutes after (crucial) the Lucozade.

INTERPRETATION

- Plasma glucose at 120 min. should be **under 7.8 mmol/L**.

glycated haemoglobin (HbA1c) monitoring 3d 2mL Daily

See ACR, fructosamine, glucose, HbA1c screen/diagnosis, **MRI** & OGTT.

Normal	27 - 48 mmol/mol
Target for control in diabetes	≤ 53
Review management	54 - 74
Significant complications-risk	≥ 75
NICE CG15 & *NICE CG87*	

Use
- Monitoring control of diabetes mellitus.
- Diagnosis. See HbA1c screen/diagnosis

Background

- Glucose reacts irreversibly with the N-terminal **valine of the β chain** of Hb type A_1 to form HbA_1c.
- Gradual formation over the rbc lifespan enables HbA1c level to reflect the "average glucose conc." in the previous **2 – 3 months** (*cf.* fructosamine 1 – 2 months).
- ↓ **rbc lifespan** eg. haemolysis, ↓ **HbA1c** for a given glucose exposure ie. **control looks better than reality**.
- **Misleading** HbA1c results can also occur in **haemoglobinopathy** (error distinguishing the various Hb peaks) & non-equilibrium states eg. **pregnancy** in 2^{nd} + 3^{rd} trimesters (NICE CG63) & **type 1 DM** at presentation.
- The Diabetes Control & Complications Trial (**DCCT**) highlighted the importance in patients with **type 1** DM of keeping HbA1c as near normal as possible in order to minimise micro & macrovascular complications. The UK Prospective Diabetes Study (**UKPDS**) drew similar conclusions in patients with **type 2** DM. See NICE CG87
- **Target HbA1c** values were identified & are given above in terms of the IFCC method & units.

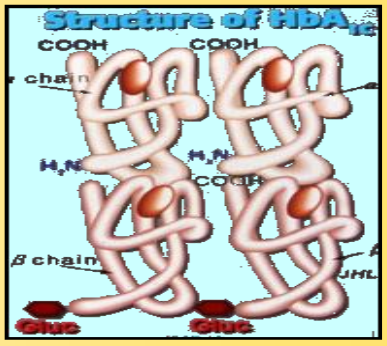
IFCC method & units (mmol/mol)

- A pure **HbA1c standard** & a **definitive method** were developed by the **International Federation of Clinical Chemistry** (IFCC) against which all HbA1c assays could be calibrated to give the same result.
- **Target figures** for management were derived by comparing the method with the one used in trials eg. DCCT
- The IFCC method & calibrant, even enable HbA1c to be measured as a **molar** quantity ie. the number of molecules of HbA1c per unit total number of HbA1 molecules: mmol of HbA1c per mol of HbA1.
- **DCCT target** 48 – 59 mmol/mol HbA1c (6.5 – 7.5 %)
- **DCCT ref. range** 20 – 42 mmol/mol HbA1c (4.0 – 6.0 %)

Women with DM who plan to become pregnant should have monthly HbA1c tests (NICE CG63).

HbA1c Conversion chart. IFCC (mmol/mol) vs. DCCT (%) concs.

DCCT	**IFCC**	*DCCT*	**IFCC**	*DCCT*	**IFCC**	*DCCT*	**IFCC**	*DCCT*	***IFCC***
5.0	**31**	*6.0*	**42**	*7.0*	**53**	*8.0*	**64**	*9.0*	***75***
.1	**32**	*.1*	**43**	*.1*	**54**	*.1*	**65**	*.1*	***76***
.2	**33**	*.2*	**44**	*.2*	**55**	*.2*	**66**	*.2*	***77***
.3	**34**	*.3*	**45**	*.3*	**56**	*.3*	**67**	*.3*	***78***
.4	**36**	*.4*	**46**	*.4*	**57**	*.4*	**68**	*.4*	***79***
.5	**37**	*.5*	**48**	*.5*	**58**	*.5*	**69**	*.5*	***80***
.6	**38**	*.6*	**49**	*.6*	**60**	*.6*	**70**	*.6*	***81***
.7	**39**	*.7*	**50**	*.7*	**61**	*.7*	**72**	*.7*	***83***
.8	**40**	*.8*	**51**	*.8*	**62**	*.8*	**73**	*.8*	***84***
.9	**41**	*.9*	**52**	*.9*	**63**	*.9*	**74**	*.9*	***85***



glycated haemoglobin (HbA1c) screen/diagnosis 3d 2mL

Daily

See ACR, fasting glucose, fructosamine, HbA1c monitoring, **MRI** & OGTT.

- **Fasting not needed.**
- **Diagnosis of DM should not be based on 1 abnormal result in the absence of symptoms.**

	HbA1c mmol/mol	**Glucose "equivalent"** (mmol/L)	
		FPG	**OGTT 2h glucose**
Normal	**< 39**	≤ 5.5	< 7.8
↑ risk (pre-diabetes)	**39 – 46**	5.6 – 6.9 (IFG)	7.8 – 11.0 (IGT)
diabetes	**> 46**	≥ 7.0	≥ 11.1
ADA 2010 & *WHO 2011*			

Background

- Glucose reacts irreversibly with the N-terminal **valine of the β chain** of Hb type A_1 to form **HbA_1c**.
- Gradual formation during the 120d lifespan of rbcs means that the HbA1c level reflects the "average glucose conc." in the **previous 2 – 3 months** (fructosamine, 1 – 2 months).
- The **American Diabetes Association** (ADA) concluded in 2010 that HbA1c assays were now good enough for it to be an alternative to plasma glucose for screening & diagnosis of **type 2** diabetes ADA 2010.
- E**ndorsed by the WHO** & Diabetes UK in Jan. 2011.
- **More convenient** than glucose – no fasting, sample at any time of day etc.
- But **much more expensive** for labs!
- **Diagnostic-concordance** between HbA1c & glucose is **not 100%** eg. the prevalence of "pre-diabetes" is lower by HbA1c than by FPG. Conclusions from fasting & 2h plasma glucose are not fully concordant either.
- **Which one is "right"?** As with results of any analyte, if they are at or near the ref. limits **diagnostic uncertainty** is more likely ie. **review clinical evidence & remedy risk factors** eg. obesity & lack of exercise, then repeat the test after at least 3 months in the case of HbA1c.

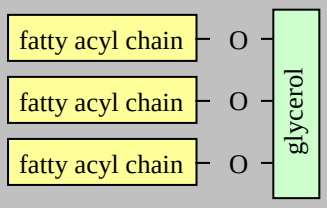
DON'T use HbA1c for diagnosis in the following conditions where misleading results may occur**:**

- pregnancy - physiological changes deceptively ↓ HbA1c.
- type 1 diabetes mellitus - hyperglycaemia can occur too rapidly to be reflected.
- haemolysis - ↓ rbc survival ↓ time for HbA1c formation ie. falsely low results.
- haemoglobinopathy - interference from abnormal forms of Hb.
- POCT systems - inadequate assay performance

glycolipid, **neutral lipid, phospholipid, sphingolipid, sphingophospholipid** and **glycosphingolipid.**
See ALP, FFA, GAGs, α-galactosidase A, Tg, vacuolated lymph, VLCFA & wbc enz.

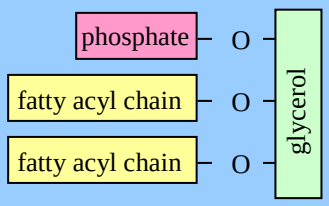
- A diverse group of compounds with **lipid** & **carbohydrate moieties in the same molecule** ie. they are **amphipathic** like phospholipids in having a hydrophobic region located in the cell membrane & a hydrophilic component (carbohydrate) which is more stable in the external aqueous environment.
- Located particularly in the **external cell membrane** & have **roles** in cell recognition eg. **blood group antigens**, **signalling** & **adhesion**. However their functions may be more subtle & complex, because knock-out of glycolipid synthesising enzymes in mice is largely without effect & disease causing mutations are unknown in humans. This contrasts with defects of glycolipid *catabolism*.
- Although glycolipids **do not have genes**, the specificity of their structure & expression is determined by the tissue specific expression of particular enzymes for their synthesis.

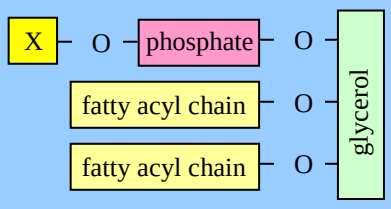
NEUTRAL LIPIDS



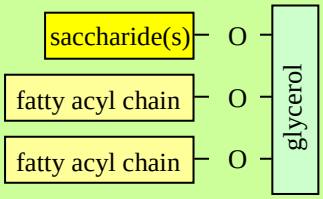
Triglyceride (triacylglycerol)

PHOSPHOLIPIDS *(glycerophospholipids or phosphoglycerides)*

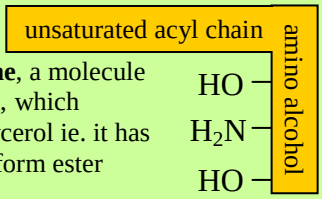




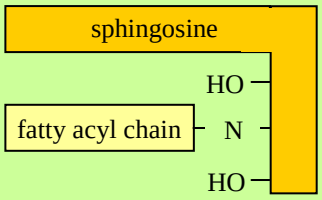
Phosphatidic acid…...……...which forms **Phosphatidyl esters** (*phosphatides*) with (X) eg. choline (*phosphatidylcholine* syn. lecithin), ethanolamine (*phosphatidyl ethanolamine* syn. *cephalin*), inositol (saccharide) & serine (amino acid). The X moiety provides further hydrophilic groups at body pH.



← **Plants & algae**, but **not humans**, can form **glycoglycerolipids** with saccharides linked directly to diacylglycerol by glycosidic bonds.

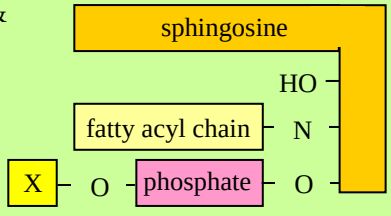


➔ **Human glycolipids** are based on **sphingosine**, a molecule with a 15 carbon unsaturated hydrophobic chain, which terminates in a 3 carbon region analogous to glycerol ie. it has 2 hydroxyl groups & 1 amino group which can form ester & glycosidic & amide linkages respectively.



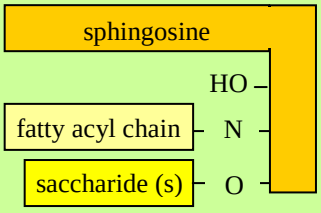
← **Ceramide,** a **sphingolipid,** is formed by amide linkage (N) of a fatty acid to sphingosine. Ceramide is actually a family of compounds with fatty acids of various lengths & saturation. It is not a glycolipid but it is a precursor:

➔ **Sphingophospholipids** are abundant & analogous to glycerophospholipids ie. ceramide esterifies with phosphoric acid which further esterifies (**X**) eg. choline (**sphingomyelin**, 25% of rbc membrane phospholipid) & ethanolamine .



HUMAN GLYCOLIPIDS

➔ **Glycosphingolipids** (formed by direct glycosidic linkage of saccharides to ceramide) are abundant in humans, unlike glycoglycerolipids (see above). Monosaccharides eg. galactose & glucose, form **cerebrosides**. These glycolipids are present in the **myelin** of peripheral nerves & CNS. Sulphation of galactosylceramide forms **sulphatide**. Oligomerisaton from an initial glucose unit eg. by adding galactose, forms **globosides** (a ceramide with 2 or more neutral monosaccharides) & **gangliosides** (the oligosaccharide contains 1 or more sialic acid residues).

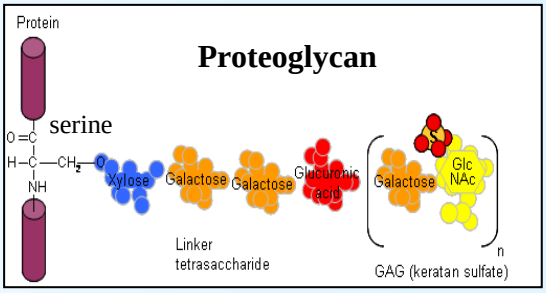


glycosaminoglycans (GAGS) 1d 5mL (mucopolysaccharides).

Sent

Refs & comment with result
Great Ormond Street Hospital

See amino acids, α-galactosidase A, glycolipid, I cell disease, organic acids, vacuolated lymphocytes, vit A, VLCFA & wbc enz.



Use • **A screen** for deficient catabolism of complex carbohydrate moieties (GAGs) in **mucopolysaccharidoses** & **mucolipidoses.**

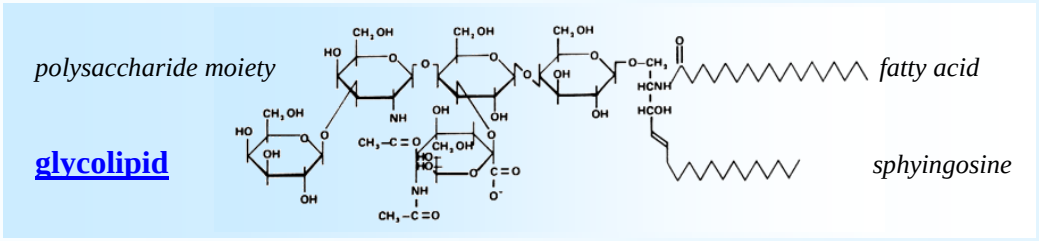
Mucopolysaccharidoses - Background

- GAGs are long **unbranched** chains (40-100+) of repeated pairs of **saccharides** & **amino sugars** eg. galactosamine, which have a high **negative charge** & **water-retaining** ability, because of the many sulphate & carboxyl groups.
- GAGs are covalently **linked to proteins by a common xyl-gal-gal** trisaccharide to form **proteoglycans** (chondroitin uniquely does not have a protein core).
- The massive & complex glycation of proteoglycans compared with glycoproteins eg. FSH, means that their properties **reflect the carbohydrate rather than the protein content**.
- Autosomal recessive deficiences of the **11 lysosomal enzymes** which break-down & recycle GAGs, cause **lysosomal-storage diseases** called **mucopolysaccharidoses** eg. Hurler & Sanfilippo syndromes, in which undegraded GAGs accumulate in lysosomes & overflow into urine. Hunter syndrome uniquely, is **X-linked**.
- The **clinical features** reflect the contribution of proteoglycans to inter-cellular matrix, connective tissue, bones, joint fluid & cartilage eg. course facies, dwarfism, axial & peripheral skeletal defects, deafness, hepato-splenomegaly, ophthalmic & neurological disorders. **"GAGs" are a screen** for these diseases.

Mucolipidoses – Background

cf. lipidoses eg. Fabry's disease).

- These lysosomal storage diseases eg. ML types 1 & 4, I-cell disease & pseudo-Hurler syn, are related to the mucopolysaccharidoses & have **similar clinical features**, but urine **GAGs are less abnormal** in ML
- The similarity arises because the defects which cause the deficiency of degradative enzyme activity in mucolipidoses, also affects the enzymes which cause mucopolysaccharidoses.
  There are defects of:
- lysosomal structure,
- targetting to lysosomes of enzymes made in the ER/Golgi
- intralysosomal environment eg. pH
- Consequently, **multiple lysosomal enzymes** are affected, which leads to ↓ catabolism of cell membrane & myelin components termed **glycolipids** (lipids, rather than proteins, with complex saccharide moieties) in addition to ↓ degradation of proteoglycans. Thus, lysosomes accumulate **glycolipids** eg. globosides & gangliosides, plus **proteoglycans** & **GAGs** as in the mucopolysaccharidoses.



growth hormone 3d 1.5mL

See GH suppr. test, glucagon stim. test, glucose, IGF1 & IGFBP3.

Sent

ug/L (ug/L x 3 = mIU/L)
Royal Surrey County Hospital

Use

- **Ix of GH secretion** in response to stimulants or inhibitors.
- **< 1y & > 75y age**, in spot samples, GH levels may reflect GH secretion better than IGF1 levels.

Test

- **Random GH results** are difficult to interpret because of large physiological variation.
- **IGF1 levels are more steady** & reflect GH function better **in spot samples**, except at the extremes of age.
- **Dynamic tests suffer less physiological interference** & are **diagnostically more sensitive & specific**. Measure GH at all time points after suppression or stimulation of secretion, but IGF1 in only the basal sample.

Background

- 191aa **single-chain peptide** secreted by the **somatotropes** of the anterior pituitary.
- Hypothalamic neurones discharge **growth hormone releasing hormone** (GHRH, 44aa) & **somatostatin** (14 & 28aa) into the hypothalamo-pituitary portal system to stimulate & inhibit GH secretion, respectively.
- **Circulating GH is mostly bound to growth hormone binding protein** (GHBP).
- GH binds to the extracellular domain of **plasma membrane receptors** which then homodimerise & signal via the JAK/STAT (Janus kinase/signal transduction & transcription) pathway.
- GH has direct effects, but **promotion of growth is via insulin-like growth factor 1** (IGF1), which is secreted in response to GH by target tissues & by the liver for export.
- **Deficiencies of growth** can result from defects anywhere along the path from:

- GH secretion
- GH receptor (Laron dwarfism)
- intracellular signalling
- IGF1 secretion
- IGF1 receptor
- IGF1 downstream signalling.

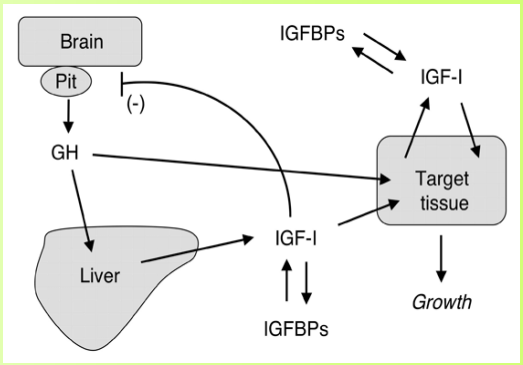
- **GH hyposecretion is commonest**.
- Defects downstream of GH are much rarer, more severe & can be syndromic. In these, GH levels can be ↑ with levels of IGF1 which are ↓ or even rarer ↑, depending on the site of the molecular lesion.
- GH **secretion is pulsatile** & subject to many influences eg.

Causes of ↑

- stress
- exercise
- sleep
- hypoglycaemia
- protein intake
- arginine
- oestradiol
- ghrelin
- GHRH

Causes of ↓

- somatostatin
- hyperglycaemia
- corticosteroids



gut hormone profile 0d 6mL + **aprotinin** Contact lab.
See CART, CgA, CgB, gastrin, glucagon, PP & VIP .
gut hormone profile part II

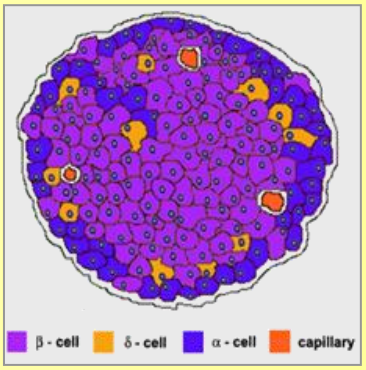
Sent

- MUST keep samples on **ice**.
- MUST be **fasting**.
- MUST supply **drug details**.
- **STOP** PPIs 14days & H2R blockers 3days before sample.
- Measure **U+E** & **Ca** too.

VIP	< 30 pmol/L
PP	< 300
gastrin	< 40
glucagon	< 50
CART	< 85
somatostatin	< 150
chromogranin A	< 60
.. .. **B**	< 150
neurotensin	< 100
Charing Cross Hospital	

Use • Ix of **gastroenteropancreatic neuroendocrine tumours** (NETs) eg. as causes of atypical peptic ulcer disease or chronic severe watery diarrhoea (> 0.5L/d) with electrolyte & acid-base abnormalities.

Background



- A **panel of neuropeptides** is tested (rather than selected ones) to confirm results & because 7% of these very rare tumours secrete > 1 product.
- These are **normal enteroendocrine peptides**, which are secreted physiologically eg. in response to food to regulate digestion & satiety
- **Fasting** & **avoidance of drugs** which ↑ levels eg. PPIs, is **crucial** to distinguishing pathological from normal levels.

VIP (Vasoactive Intestinal Polypeptide).

- 28aa peptide released from **gut nerve termini**, which relaxes gut smooth muscle, dilates vasculature, inhibits gastric acid production & stimulates bicarbonate, water & electrolyte secretion by the pancreas & gut mucosa.
- VIPomas are v. rare. **90% arise in the pancreas** (75% in the tail). Other sites: bronchogenic ca., colonic ca., phaeochromocytoma, hepatoma & adrenal tumours. Children may get VIPomas in sympathetic ganglia & the adrenal medulla.
- Excess secretion of VIP causes **Verner-Morrison** syn.: Wt loss, severe watery diarrhoea without steatorrhoea (80% >3L/day. **<0.7L/day excludes the diagnosis**), dehydration, hypokalaemic acidosis, hypercalcaemia (PTHrP secretion), hyperglycaemia, flushing, weakness & hypotension
- **> 50 %** of **VIPomas are malignant** & 6% are part of the **MEN1** syndrome.

PP (pancreatic polypeptide).

- 36aa peptide secreted by **pancreatic F (PP) cells** in the islet periphery.
- Function unclear. ?Co-ordination of digestion. PP has a **biphasic effect**, initially stimulating & then suppressing pancreatic secretion of water, enzymes & electrolytes. PP also stimulates gastric emptying & gut motility.
- **PP levels ↑ for 4 – 8h after food**. Also ↑ with prolonged fasting, type 1 DM, exercise, vagal stimulation, carcinoids & DU. ↓ levels can be seen in pancreatitis & pancreatic insufficiency.
- **All PPomas are pancreatic**, very rare & can present like VIPomas with **V-M syndrome**.
- **> 60% are malignant** & most are silent & present with abdo. pain, Wt. loss, hepatomegaly & jaundice.
- Plasma PP more often ↑ in association with other NETs eg. pancreatic VIPoma, glucagonoma, gastrinoma & insulinoma. **20 – 40% of cases are associated with MEN1**.

• **Gastrin** See gastrin. Sample – Li hep., aprotinin & ice are needed if gastrin is part of a gut hormone profile.

• **Glucagon** See glucagon.

See gut hormone profile part II ➡

gut hormone profile part II

CART (Cocaine & Amphetamine Regulated Transcript)

- **Stable in plasma** at room temp without aprotinin
- Levels are **unaffected by age, gender or time of day**
- 1st seen in rat brain as ↑ of its **mRNA in response to cocaine**.
- CART protein is expressed in the **central, peripheral** & **enteric nervous systems** & **pancreas**.
- It has many complex roles eg. **islet regulation** & behaviour related to reward, satiety, stress & addiction. It might even mediate comfort eating eg. ↓ brain CART in depression → ↑ appetite. *CART* alleles link to alcoholism.
- ↑ plasma CART is seen with **NETs of all types**, but especially pancreatic ones.
- Combined CART, CgA & CgB tests, give a > **90% sensitivity for pancreatic NETs**. Bech P et al 2008.
- Other causes of ↑: ↓ **GFR** & a few % of patients with carcinoma of **prostate, colon & pancreas**..

Neurotensin (NT)

- **REPLACED BY CART ASSAY** because CART results are more sensitive for NET detection.
- NT is a 13aa neuropeptide expressed in the **brain & gut**. In the hypothalamus it has roles in:

- dopaminergic neurotransmission
- regulation of LH & prolactin secretion.

- In the gut, highest levels are present in the **ileum & jejunum**.
- NT has roles in:

- gut regulation eg. causes ↓ gastric emptying & ↑ intestinal transit.
- feedback to the brain on food intake
- inhibition of gastrc acid secretion
- regulation of pancreatic secretion of insulin, glucagon, somatostatin, PP & digestive juice.

- **Pure neurotensinomas are v. rare** & can present with oedema, cyanosis, hypotension & flushing, rather than diarrhoea.
- NT assay is **still available** for specific uses eg. fibrolamellar hepatic carcinoma.

Somatostatin

- 14aa peptide secreted by the **pancreatic D (δ) cells** in the periphery of the islets.
- **Secretion is ↑ by high fat, carbohydrate** & **protein** rich meals.
- **Inhibits** a wide range of pancreatic & intestinal functions eg.

- gut motility
- gallbladder contraction
- gastrin secretion
- insulin & glucagon secretion

- **Somatostatinomas are v. rare**: 55% are **pancreatic** (70% in head) & 45% are **duodenal** or **jejunal**
- 50% of gut ones are associated with **neurofibromatosis type 1**.
- **60%** of somatostatinomas are **malignant** & up to 80% have metastasised at diagnosis.
- Only 10% of patients manifest the classic syndrome of **diabetes, steatorrhoea & gall stones**.
- **Wt.↓** & **abdo. pain** the commonest symptoms.

Chromogranin A See chromogranin A

Chromogranin B (secretogranin 1). See chromogranin B

H

haptoglobin 3d 5mL

Sent

See bilirubin & hemopexin.

Women	0.4 – 1.6 g/L
Men	0.5 – 2.0
Sheffield Protein Reference Unit	

Use

- detection of intravascular haemolysis.

Background

- **Free Hb is toxic**.
- Haptoglobin binds free Hb & transports it to the **reticulo-endothelial cells** which recycle the iron & protein.
- The **acute phase response** ↑ haptoglobin levels, which counters the ↓ due to haemolysis
  ie. **inflammation ↓ sensitivity for detecting haemolysis** (inflammation & haemolysis often coexist)

Causes of ↓

- haemolysis – haptoglobin levels ↓ when it combines with free Hb.
- haemoglobinopathies
- acute + chronic hepatitis
- oestrogen eg. OCP, pregnancy

Causes of ↑

- inflammation
- corticosteroids
- cholestasis

hCG (human chorionic gonadotropin) 3d 1.5mL

Daily

See AFP, CA125, CA153, CA199, CEA, FSH, LH, **MRI**, oestradiol, PLAP, progesterone & prolactin.

- **Not for Down's screening.**

Use

- Detection & monitoring of pregnancy
- In Oncology

Background

- Differences in assay-specificity for hCG fragments makes it **essential to use the correct ref. range.** This assay measures:

- intact hCG
- nicked hCG (partially cleaved β-chain)
- β-core fragment (degraded βchain residue)
- free β-subunit.

Can be used in **pregnancy** & **oncology** eg. hydatidiform mole & gonadal & extragonadal tumours with trophoblastic elements in **females** & **males**.

- hCG is a glycoprotein **heterodimer** structurally & functionally **like LH**. Its β chain is unique but the α-chain is the same as in TSH, FSH & LH.

- Synthesised by the **syncitiotrophoblast** of the placenta, hCG becomes detectable in serum **1w after implantation**. Levels double every 36 – 48h up to 6/40 then slow to peak at 8 – 13/40. The wide range of values, even in health, makes hCG **unsuitable for dating**.

Gender	Physiology	**hCG** (IU/L)
Women (non-pregnant)	**pre**-menopause	≤1.0 *(97.5th centile)*. Upper 95% conf. limit of this = 5.3
	post-menopause	≤7.0 *(97.5th centile)*. Upper 95% conf. limit of this = 8.3
Women (pregnant)		*(5th – 95th centiles)*
	3 weeks	5.8 - 71
	4	9.5 - 750
	5	217 - 7,138
	6	158 - 31,795
	7	3,697 - 163,563
	8	32,065 - 149,571
	9	63,803 - 151,410
	10	46,509 - 186,977
	12	27,832 - 210,612
	14	13,950 - 62,530
	15	12,039 - 70,971
	16	9,040 - 56,451
	17	8,175 - 55,868
	18	8,099 - 58,176
Men		≤ 2.0 *(97.5th centile)*. Upper 95% conf. limit of this = 2.6
Roche Cobas HCG+β method sheet V16		

- The serum **half-life is 18 – 24 h**.

- hCG binds to ovarian LH receptors to **stimulate & maintain progesterone production by the corpus luteum** until placental progesterone secretion starts to take over at about 5 w gestation. Accompanying the ↑ serum hCG of pregnancy, there is thus, ↑ oestradiol, ↑ progesterone, ↓ FSH & ↓ LH (especially).

HDL (high density lipoprotein) cholesterol 3d See profiles

See cholesterol (total), cholesterol:HDL ratio, LDL, **MRI** & Tg.

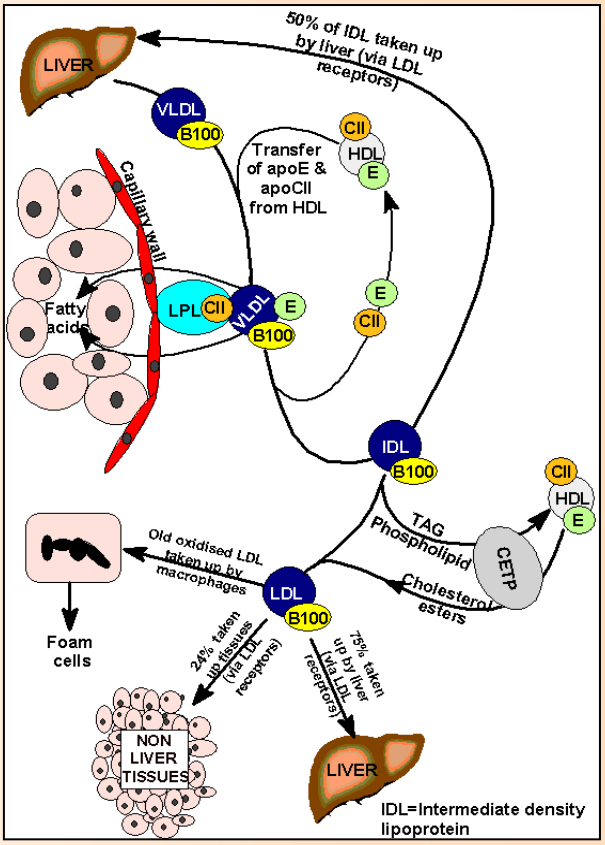
gold Daily

Use

- **Estimation of CVD risk** when combined with total chol. level & other risk factors.

	Minimal risk	**Moderate** risk	**Higher** risk
Women	>1.68	1.15 - 1.68	<1.15 mmol/L
Men	>1.45	0.90 - 1.45	<0.90 mmol/L
Roche Cobas HDLC3 method sheet V3			

NCEP CHD risk guideline values:
high risk……..…≤1.04 mmol/L
negative risk...…≥1.56



Background

- HDL cholesterol represents **"reverse-transport"** cholesterol ie. from tissues ➔ liver.
- Levels are **inversely related to CVD risk** & counter the effect of LDL (low density lipoprotein cholesterol, hence the importance of the total cholesterol/HDL cholesterol ratio for CVD risk estimation.
- **Ultracentrifugation** of plasma reveals protein & lipid complexes which can be categorised by their density, which reflects the **protein** content:

- HDL is 55% protein & the **most dense**
- VLDL is 10% protein, 90% lipid & the least dense.

These proteins (**apoproteins**) control functions eg.:

- lipoprotein secretion (A1)
- tissue uptake (A1)
- structure & the activation of enzymes (A1 & CII).

HDL biochemistry is intricate

- **Nascent HDL** is secreted by the liver & intestine. This readily **takes up cholesterol** from tissues. When the plasma enzyme **LCAT** (*lecithin cholesterol acyl transferase*) is activated by the **apo A1** in these HDL precursors, it esterifies their cholesterol with fatty acids from the phospholipid content.
- Some esterified cholesterol remains in HDL & some is exchanged by **CETP** (*cholesterol ester transfer protein*) for triglyceride in VLDL, chylomicrons & intermediate density lipoprotein (IDL), which becomes LDL.
- This Tg is removed by **hepatic triglyceride lipase** during circulation through hepatic capillaries.
- **HDL is taken up by the liver** or can rejoin the HDL pool to receive more cholesterol & Tg.

HDL is not the only path back to the liver:

- Cholesterol enters the liver by **receptor mediated** binding of 1) **HDL** (apoA1) 2) **chylomicron remnants** (apo E) & 3) **IDL** & **LDL** particles (apo B100) derived from VLDL supplemented with cholesterol by CETP. However, unlike these other liporoteins, **HDL does not deliver cholesterol to peripheral tissues**.

Causes of ↑

- exercise
- modest ethanol intake
- reduction of ↑ Wt.
- control of diabetes
- reduction of ↑ Tg levels
- fibrates
- PBC
- chronic hepatitis
- polygenic inheritance
- familial hyper-α-lipoproteinaemia

Causes of ↓

- uncontrolled diabetes
- hepatitis
- cholestasis
- nephrosis
- CRF
- familial hypo-α-lipoproteinaemia
- abeta-lipoproteinaemia eg. genetic defects of apo AI, apo CII & LCAT.

hemopexin 3d 5mL

See bilirubin, haptoglobin & porphyrin.

Sent

Women	0.6 – 1.3 g/L
Men	0.5 – 1.1
Sheffield Protein reference Unit	

Use

- Detection of intravascular **haemolysis.**

Background

- A **70 kDa glycoprotein** secreted by the **liver**.
- Binds **free haem** released by intravascular haemolysis (4 haem/Hb molecule, see porphyrin) & transports it to the **liver** where hemoxin is released & resecreted, iron is recycled & the rest of haem disposed of as bilirubin.
- *cf.* **haptoglobin** which binds free Hb & the whole complex is catabolised by the reticuloendothelial system.
- **Haem is toxic** (generates free radicals) & can enter the glomerular filtrate which would be a waste of iron.
- The **balance of hemopexin synthesis & uptake by the liver** determines the normal serum level.
- ↑ **haemolysis** accelerates removal → ↓ serum hemopexin.
- Hemopexin is **less affected by inflammation** (the acute phase response) than haptoglobin.
- Assay of **both** is recommended for Ix haemolysis.

Cause of ↓

- intravascular haemolysis

Cause of ↑ (< 2 fold)

- acute phase response

hydroxycarbamazepine (MHD) 3d 1.5mL

See carbamazepine, oxcarbazepine & therapeutic drug monitoring.

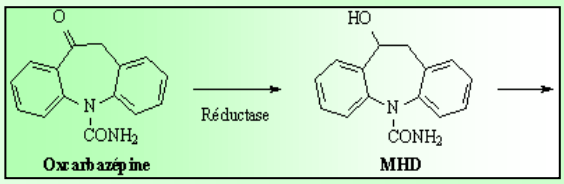
Sent

15 – 35 mg/L
St. Thomas' Hospital

Use

- **Routine monitoring** of oxcarbazepine/MHD is **unnecessary** because of the predictable pharmacokinetics ie. the dose is an adequate guide.
- But, **levels may be useful in**:

- ↓ **GFR** (30% of MHD is excreted unchanged in urine)
- **multiple drug therapy** (phenytoin & phenobarbitone cause ↓ MHD levels)
- Ix of **compliance** or loss/lack of clinical effect
- Ix of **toxicity**.



Background

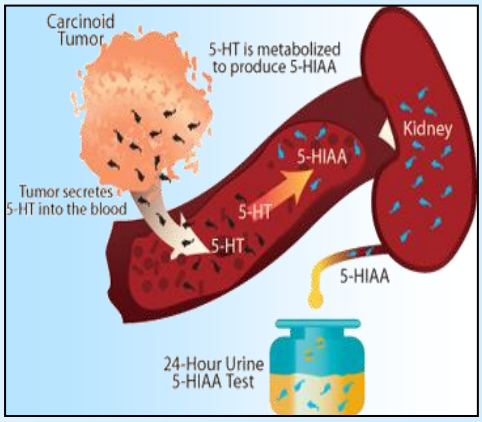
- Oxcarbazepine is a **derivative of carbamazepine** with ↓ severe side effects eg. hepatoxicity & agranulocytosis.
- Like carbamazepine, oxcarbazepine inhibits Na channels & is used to treat **epilepsy & bipolar disorder**.
- Oxcarbazepine is **well absorbed** & **rapidly reduced** (half-life **2h**) by hepatocyte cytosolic enzymes to hydroxycarbamazepine (monohydroxy derivative, MHD) (half-life **19h**, inactivated by conjugation).
- **MHD accounts for most of oxcarbazepine's activity**.
- Thus, oxcarbazepine levels are **actually reported as MHD levels**.

5-hydroxyindole acetic acid (5HIAA) 3d 24h urine
See aldosterone, CgA, renin & VMA.

Sent

< 47 umol/24h
St Helier Hospital

For **children** & adults unable to collect 24h urine.
Reported as 5HIAA/creat. ratio. **24h sample is better**.



Use • Diagnosis & monitoring of carcinoid neoplasms.

Sampling

- **Protect from bright light**
- **Keep cool**.
- The lab. will add **acid** preservative on delivery of the sample
- **x2** 24h collections are sufficient for exclusion.
- Urine collected after a **symptomatic event** is especially useful.
- **Procedure**: Urinate in the toilet & note the time (can be any time). Collect all urine for the next 24h. The last one to collect is whatever can be passed at the noted-time next day.

Interference. AVOID:

- **Food** high in 5HT (5-hydroxytryptamine, serotonin) eg. walnuts, plums, bananas, pineapple, kiwis, avocado, tomatoes. Avoid these for **48h before the start & during the collection.**
- **Tea & coffee**. Avoid **during the urine collection –** stimulate secretion of 5HT.
- **Smoking**. Avoid **during the urine collection –** stimulates secretion of 5HT
- **Drugs**. **Give details** & ***ideally*** stop 48h before urine collection.
  ↓ results – fluoxetine, ranitidine, imipramine, methyldopa, ethanol.
  ↑ results – cisplatin , fluorouracil, melphalan.

Background

- Carcinoid tumours **secrete** bioactive substances eg. **5HT** (5-hydroxytryptamine, serotonin), **histamine** & **kallikrein** which produce characteristic **symptoms & signs**:

- **flushing** (80% of cases) for 2-5 min (can be hrs with bronchial carcinoid) & spontaneous or evoked eg. by eating
- **diarrhoea** (85% of cases), watery
- **bronchospasm** (20%)
- **abdominal pain** (40%) may coincide with flushing

- Carcinoids are tumours of the **diffuse neuroendocrine system**, which are classified by region**:**

- **foregut c.**: bronchus, stomach, duodenum, pancreas, thymus. Less common than other carcinoids & more often secrete **5-hydroxytryptophan** (5HTP) rather than 5HT.
- **mid-gut c.**: jejunum, ileum, ascending colon.
- **hindgut c.**: transverse & distal colon & rectum.

- The **appendix** is the commonest site & the tumour is usually benign & often found coincidentally.
- 5HT is **metabolised** to inactive **5HIAA** by the enzyme **monoamine oxidase**, especially in the **liver**.

Interpretation

- **Presention** with the carcinoid syndrome is **frequently associated with metastasis**.
- **Absence of symptoms does not exclude** carcinoid or metastases - tumour function & bulk might be low.
- High urinary 5HIAA suggests carcinoid, but levels may be normal or only marginally ↑ with **localised tumours**.
- **Chromogranin A** assay is **more sensitive** than 5HIAA for detecting carcinoids, but **less specific**.

homocystine (plasma) 0d 6mL

See cystine, folate (serum), folate (rbc), vit B6, homocystine (urine), methylmalonate & vit. B12.

Sent

5 – 15 umol/L (fasting)
Royal Sussex Univ. Hospital, Brighton

- **FASTING.**
- Take blood at **ESH or CH**.
- Plasma MUST be separated from cells < 4h.

Homocystine	Methylmalonic acid	Interpretation
↑	↑	Compatible with vit. B12 def.
normal	normal	Vit. B12 deficiency unlikely.
↑	normal	Folate deficiency possible
Interpretation if serum methylmalonic acid available too. Ditto		

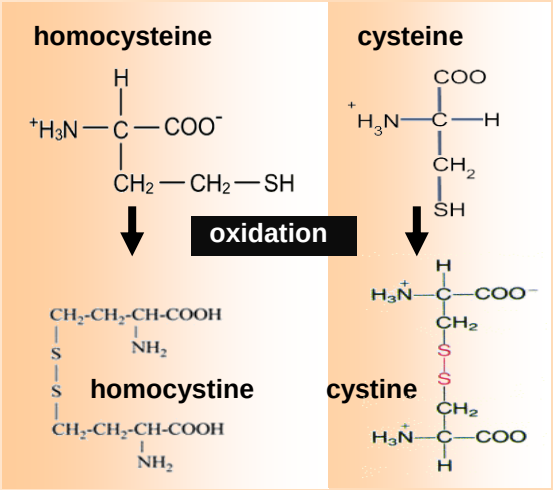
Use Ix:

- Homocystinaemia.
- Unclear folate & vit B12 results.

Background

- An **α-amino acid** **metabolic intermediate**. Not in proteins.
- **Similar to cysteine**:

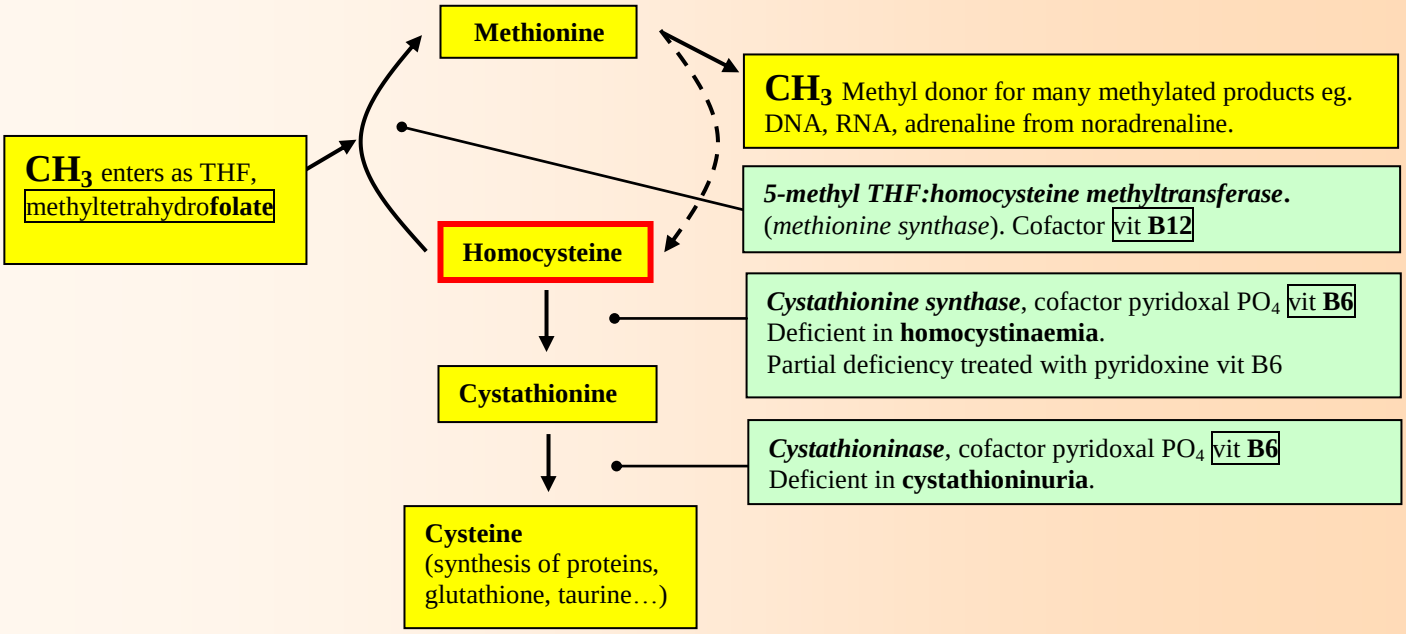
- but R group is 1 methylene (CH_2) longer.
- 2 homocysteines **readily oxidise** to form a disulphide bond & **homocystine**. *cf.* 2 cysteines forming cystine:



Causes of ↑

- **Homocystinaemia**. 1:45 000, autosomal recessive, mutations of the gene for ***cystathionine synthase***. Neurodevelopmental delay, mental retardation, skeletal abnormalities (similar to Marfan's syn.) lens dislocation, ↓ pigmentation, thromboembolism & CVD in children & young adults. Varied phenotype eg. a milder, vit. B6 responsive form. Plasma homocystine ↑, methionine ↑ + cystine ↓. **Therapy**: ↓ methionine diet plus folate & vit. B6 supplements.
- Heritable defects of ***methionine synthase*** & cofactor synthesis can also cause homocystinaemia.
- Mild ↑ in the general population correlates with ↑ CVD risk but treatment to ↓ levels is without benefit
- **Folate** & vit **B12 deficiency**: plasma homocystine ↑, but NOT methionine (see below). Vit. B12 def. also ↑ serum **methylmalonic acid** (vit. B12 is a cofactor).

Folate & vit. B12 deficiency cause ↓ cofactor function & ↑ plasma homocysteine



homocystine (urine) 0d 20 mL

See cystine, folate, homocystine (plasma) & vit. B12.

- Assay of **plasma homocystine is better.**
- **False + ves** due to **bacterial** synthesis of homocystine from normal urinary cystathionine, may occur.

17-hydroxyprogesterone (17OHP) in blood-spots **0d** **Guthrie card** Sent

See cortisol, 17OHP (serum) & renin.

See report. nmol/L
Univ. Hosp. Southampton

Use

- Monitoring CAH therapy in children with difficult venous access eg. for a day-profile.

Test

- Apply drips of capillary blood to the card as in neonatal screening.

17-hydroxyprogesterone (17OHP) 3d 1.5mL

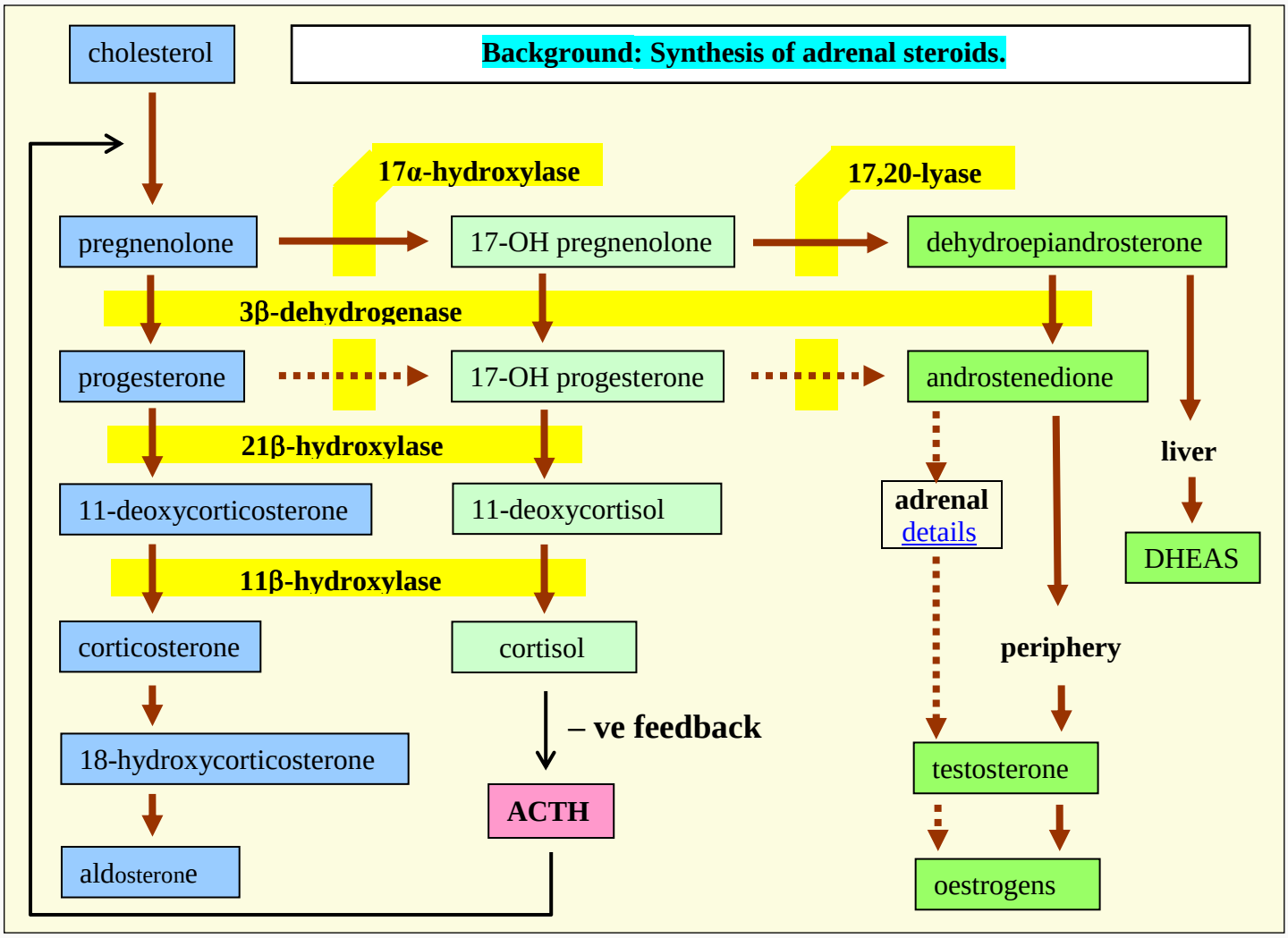
See aldosterone, androstenedione, cortisol, 11-deoxycortisol, DHEAS, DHT, 17OHP (blood spot), renin, SST, steroid profile, testosterone & virilisation.

Sent

1 – 10 nmol/L
St Helier Hospital

Use

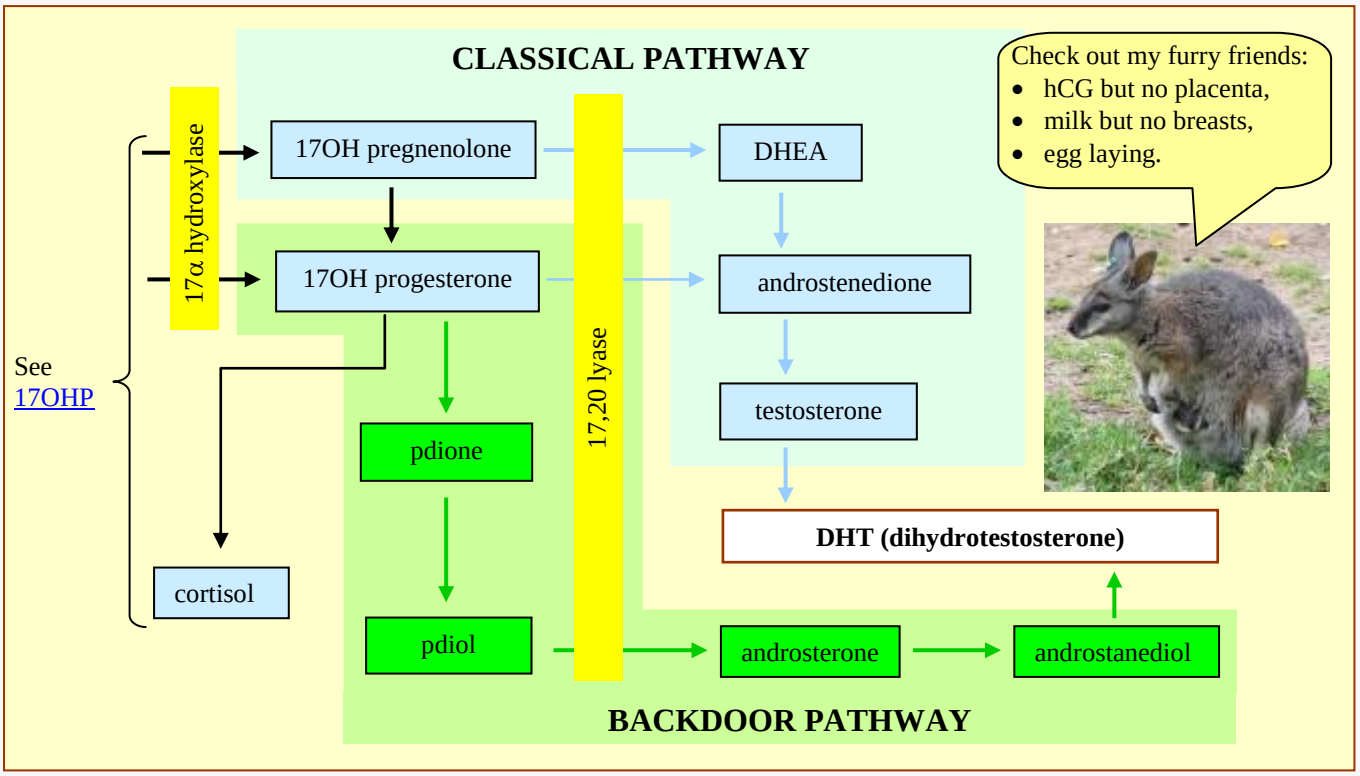
- Ix of **congenital adrenal hyperplasia** (CAH). **Sample after 3rd postnatal day**, if poss.
- **Follicular phase samples** give fewer false + ve 17-OHP & androgen results.



- In **CAH**, deficiency of enzymes catalysing cortisol synthesis, cause ↓ inhibitory feedback by cortisol on the pituitary, ↑ ACTH secretion & hyperstimulation of the biochemical pathway. Substrate(s) upstream of the deficient enzyme accumulate & divert into alternative syntheses. See virilisation.
- **21-hydroxylase deficiency** (90% of cases) ↑ serum 17-OHP. **Gross loss-of-function** mutations disable **aldosterone synthesis** too, leading to **urinary "salt-loss"** & **hypotension**.
- **11-hydroxylase deficiency** (5% of cases) ↑ serum 17-OHP **and 11-deoxycortisol** too. But **no salt loss**: actually **salt retention** & **↑BP** because accumulated **11-deoxycorticosterone** has mineralocorticoid activity.
- Both defects ↑ **androgen levels** – testosterone, dehydroepiandrosterone sulphate (DHEAS) & androstenedione, causing **virilisation** of females, **hirsutism**, **menstrual disorders** & **precocious puberty** in both sexes. **BUT…**
- **Less defunctioning mutations** cause clinical abnormalities which are **milder & present later**.
- A Synacthen test exaggerates the biochemical effects of CAH., which may clarify borderline results.
- Normal **neonates**, especially premature & stressed ones, can **have apparently high 17OHP levels** due to assay cross-reaction with naturally high levels of pregnenolone & 17-hydroxypregnenolone sulphate at this age, but they **rapidly fall** within 2 - 3 days of birth.
- A **urine steroid profile** examines dozens of steroids & metabolites simultaneously ie. if **genital sex is unclear,** it can distinguish CAH from the complexities of normal in week 1 more certainly than isolated assays so that the distressed parents can be given an answer asap.

Androgens & virilisation in CAH The backdoor pathway.

See 11-deoxycortisol, DHEAS, DHT, 17OHP & steroid profile.



Comparative biology & rare CAH cases reveal complexity in androgen synthesis & virilisation of girls.

Classical pathway for testosterone synthesis

- **From 17 hydroxypregnenolone** via DHEAS & androstenedione, to testosterone in gonads & adrenal cortex.
- **17 hydroxyprogesterone (17OHP) enters this path too**, as androstenedione.
- However, **human *17,20 lyase* actually has little activity for converting 17OHP to androstenedione** although this can be countered by mass action of high 17OHP levels.
- Virilisation of external genitalia needs androgen of high potency ie. **DHT**. The other androgens (including testosterone) are inadequate unless converted to DHT: witness the female phenotype of XY males with complete deficiency of the enzyme *5α-reductase*, which catalyses this crucial step.

Backdoor pathway

- In 2002, a novel **pathway for synthesis of DHT from 17OHP without testosterone**, was found in wallaby testes & subsequently in the human adrenal cortex & liver.
- A **landmark study** of *21-hydroxylase* deficient patients, **confirmed** ↑ classical pathway conversion of 17OHP to androstenedione & clearly revealed ↑ androgen synthesis in the new backdoor pathway too (Kamrath 2012).
- **Entry of 17OHP into the backdoor pathway is favoured** by:
  1) low affinity of ***17,20 lyase*** for 17OHP at the start of the classical pathway.
  2) high affinity of ***5α-reductase*** for 17OHP at the start of the backdoor route.
  3) **pdiol** is the preferred substrate of 17,20 lyase, over 17OH pregnenolone.
- The **backdoor pathway has a normal role** in male development in addition to the classical pathway, because deficiencies cause inadequate androgenisation of boys eg. hypospadias (Miller 2012).

Clinical

- It is not surprising, therefore, that serum **DHEAS, androstenedione & testosterone are weak markers of CAH** & do not correlate clearly with the degree of virilisation.
- Backdoor pathway markers eg. pdiol, are **likely to enter clinical practice** in the future.

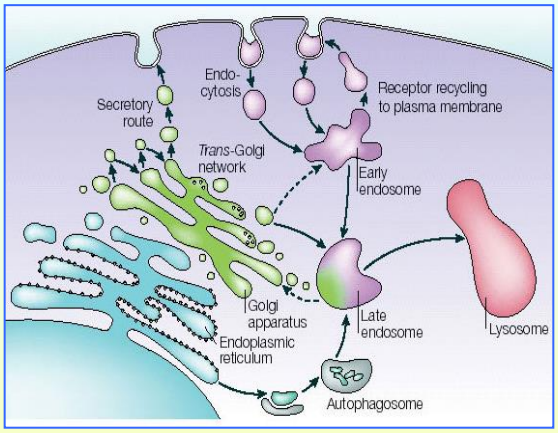
I

I-cell disease screen 0d 3mL
See Cg A, GAGs, vacuolated lymphocytes & wbc enzymes.

Sent

Refs & comment sent with result
Great Ormond Street Hospital

- **MUST** be in ESH lab. by **10am.**
- **MUST** get to GOSH by **2pm.**
- **DO NOT** send on **Friday**.
- **Invalidated** by blood transfusion in previous 6w.



Background

- I-cell disease is a **lysosomal storage disease** (type II mucolipidosis) in which the absence of **several enzymes** from lysosomes, causes their substrates to accumulate.
- **Rare** ie. approx. 1:600,000
- Named after the **visible cytoplasmic inclusions** (vacuoles) of undegraded substrates, especially in fibroblasts.
- Caused by autosomal recessive mutations of the enzyme **N-acetylglucosamine-1-phospate transferase**.
- This Golgi apparatus-resident, **phosphorylates mannose** to form mannose-6-phosphate in the carbohydrate chains of N-linked **glycoprotens** (carbohydrates linked to the side-chain nitrogen of the amino acid asparagine).
- **Mannose-6-phosphate is a crucial signal for targetting** to lysosomes of newly synthesised enzymes from the ER/Golgi apparatus.
- Without this signal, these enzymes enter the secretory pathway instead & are found at **↑ levels in plasma**.
- Thus, **this screen uses plasma rather than WBCs** as for other lysosomal storage disorders.
- There are clinical similarities with the **mucopolysaccharidoses** eg. Hurler's syndrome (not surprising since the enzymes in these conditions may be affected in I-cell disease too).
- However, the **urine** concentration of glycosaminoglycans (**GAGs**, characteristic complex linear polysaccharides) in I-cell disease is **normal or less ↑** than in the mucopolysaccharidoses.

IGF-1 (Insulin like Growth Factor 1) 1d 1.5mL

Sent

See comments, GH, GH suppr. test, IGFBP3 & glucagon stim. test.

Age yrs	**IGF-1** nmol/L
0 - 6	4 - 20
7 - 9	7 - 40
10	12 - 50
11	17 - 60
12	20 - 85
13	23 - 90
14 - 16	30 - 90
17 - 20	23 - 70
21 - 40	13 - 50
41 - 60	9 - 40
> 60	6 - 36
Royal Surrey County Hosp	

Use

- Ix of disorders of growth & GH secretion

Background

- IGF-1 is **secreted in response to growth hormone** (GH) & mediates its actions. Large quantities of IGF-1 enter the circulation from the liver & a smaller amount is produced locally in target-tissues.

- Plasma **IGF1 levels are more stable than those of GH**, which makes it **more suitable in spot samples**, than GH itself for assessing GH secretion. The stability also means that IGF-1 needs to be measured **only in the basal sample** in dynamic endocrine tests, because it will not change on the timescale of the subsequent samples.

- Circulating IGF-1 is associated with a number of **binding proteins** whose levels are related to that of IGF-1, notably IGF binding protein 3 (IGFBP3), which ↑ IGF-1 function by protecting it from proteases. IGF BP3 is a useful accompaniment to IGF-1 in the Ix of ↓ growth in **children**.

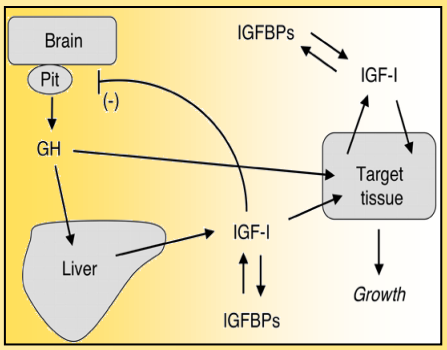
- Ref. range is **age related**. At the extremes eg. over 75y & under 1y, GH may be more informative than IGF1, but the uncertainty caused by the physiological variability of GH remains a weakness.

Causes of ↓

- hypopituitarism
- hypothyroidism
- malnutrtion
- GH deficiency
- Laron dwarfism (GH↑)

Causes of ↑

- acromegaly
- pregnancy



IGF-Binding Protein 3 (IGF BP3) 1d 1.5mL

Sent

See GH, GH suppr. test, glucagon stim. test & IGF-1.

Use • Ix of growth disorders in children.

Background

- IGF-BP3 plasma levels, like those of IGF1, are more stable than those of growth hormone & thus more suitable for gauging GH secretion from random or spot samples, especially in **children**.
- IGF-BP3 is one of **six binding proteins** which associate with **insulin like growth factors** I & II. They have complex roles in transport & inhibition and ↑ of IGF action eg. plasma IGF BP3 **protects IGF1** from degradation by proteases, thus **IGF-1 function is ↑**.

Causes of ↑

- acromegaly
- gigantism
- GH replacement (relative to previously)

Causes of ↓

- hypopituitarism
- GH deficiency

Age (y)	**IGFBP3** (mg/L)
0 – 2	0.5 – 2.9
3 – 4	0.8 – 3.4
5 – 6	1.0 – 3.8
7 – 8	1.1 – 4.3
9 – 10	1.3 – 4.6
11 – 12	1.6 – 5.0
13 – 14	2.1 – 5.3
15 – 16	2.5 – 5.4
17 – 18	2.4 – 5.4
19 – 20	2.3 – 5.3
21 – 40	1.7 – 5.2
41 – 60	1.3 – 4.8
61 – 80	0.7 – 4.4
> 80	0.5 – 4.3
Royal Surrey County Hospital	

IgG subclasses 3d 1.5mL

Sent

See BJP, FLC, immunoglobulins & serum protein electrophoresis.

Use

- Ix of patients with **recurrent infections** & a normal total IgG level.

Background

- The 4 subclasses of immunoglobulin G (types of IgG molecule) circulate at different levels which vary with age, gender & alleles.
- Responses to microbes:

- **viruses**: mainly IgG1 & 3
- **parasites**: IgG4
- **bacterial** polysaccharide: IgG1 in children & IgG2 in adults.

- **Subclass deficiency & clinical phenomena correlate poorly & inconsistently**.
- Absence of all subclasses can be without ill-effect.

	SUBCLASS (g/L)			
AGE	**IgG1**	**IgG2**	**IgG3**	**IgG4**
cord blood	3.6 – 8.4	1.2 – 4.0	0.3 – 1.5	≤ 0.5
0 - 6 m	1.5 – 3.0	0.3 – 0.5	0.1 – 0.6	≤ 0.5
6m - 2 y	2.3 – 5.8	0.3 – 3.9	0.1 – 0.8	≤ 0.5
2 - 5 y	2.3 – 6.4	0.7 – 4.5	0.1 – 1.1	≤ 0.8
5 - 10 y	3.6 – 7.3	1.4 – 4.5	0.3 – 1.1	≤ 1.0
10 - 15 y	3.8 – 7.7	1.3 – 4.6	0.2 – 1.2	≤ 1.1
Adult	3.2 – 10.2	1.2 – 6.6	0.2 – 1.9	≤ 1.3
% of total	*60 – 70*	*14 – 20*	*4 – 8*	*2 – 6*
Sheffield Protein Reference Unit (5th – 95th centiles)				

IgG1
- is so abundant that deficiency of lesser subclasses has little impact on total IgG.
- deficiency often affects other Ig too, in a form of common variable immunodeficiency.

IgG2
- the **commonest subclass deficiency in children** (1:1000).
- combined IgG2 & 4 def. is associated with ataxia telengiectasia, pyogenic infections, idiopathic, bronchiectasis, IgA def. & SLE. Total IgG may be normal because of compensatory ↑ IgG1.

IgG3
- higher in females.
- deficiency of IgG3 & IgG1 may occur together & cause **recurrent respiratory infections**.
- the **commonest subclass deficiency in adults.**

IgG4
- higher in males.
- isolated deficiency is v. rare.
- ↑ in **CF & atopy**, like IgE.

immunoglobulins 3d 1.5mL

Daily

See BJP, FLC, IgG subclasses, methylmalonate, **MRI** & serum protein electrophoresis.

		IgA (g/L)	IgG (g/L)	IgM (g/L)
Children	**0 - 1 y**	0.00 - 0.83	2.32 - 14.11	0.00 - 1.45
	1 - 3 y	0.20 - 1.00	4.53 - 9.16	0.19 - 1.46
	4 - 6 y	0.27 - 1.95	5.04 - 14.64	0.24 - 2.10
	7 - 9 y	0.34 - 3.05	5.72 - 14.74	0.31 - 2.08
	10 - 11 y	0.53 - 2.04	6.98 - 15.60	0.31 - 1.79
	12 - 13 y	0.58 - 3.58	7.59 - 15.49	0.35 - 2.39
	14 - 15 y	0.47 - 2.49	7.16 - 17.11	0.15 - 1.88
	16 - 19 y	0.61 - 3.48	5.49 - 15.84	0.23 - 2.59
Adults		0.7 - 4.0	7.0 - 16.0	0.4 - 2.3
Roche Cobas method sheets IGA-2 V8, IGG-2 V9, IGM-2 V7.				

Background & causes of ↓

Primary (congenital) deficiencies:

- Commonest (1:700) is of **IgA** & causes:
  - coeliac disease
  - infections of the sinuses & lungs
  - adverse reactions to blood transfusions & products due to anti IgA antibodies.

  Most deficient people are **symptom-free**.
- Deficiencies may present in children as**:**
  - recurrent infections (especially pyogenic, pneumococcal & Hib) affecting mucosae, skin & respiratory tract.
  - atopy
  - gut disease
  - ataxia telangiectasia.
- **Secondary deficiencies** (acquired) are **more common** eg. due to eg.:

Causative process	Examples of disease	Notes
Bone marrow disease	hypoplasia	↓ IgG especially
	neoplastic infiltration	eg. metastases, myeloma
↓ Ig survival	nephrosis	
	protein-losing enteropathy	
	myotonic dystrophy	
Toxic factors	chronic uraemia	IgM ↓ more than IgA & IgG
	sepsis	
Neoplasia	B-cell neoplasia eg. myeloma	

The causative disease may be clinically apparent & immunodeficiency predictable eg. myeloma.

- **IgD** assay may be useful in Ix **recurrent PUO** in children possibly due to **HIDS** (Hyper IgD Syndrome) a variant of **mevalonic aciduria**.
- **Paraproteins** are quantified by protein electrophoresis rather than immunoassay as with the above, because the neoplastic immunoglobulin may have atypical immunoreactivity.

immunoreactive trypsin Guthrie card

Sent

See sweat chloride.

- Heel prick blood at **1 – 2 w of age.**

<60 ug/L
Addenbrooke's Hospital

Use

- A **screen for cystic fibrosis** (CF) in neonates.

Background

- In CF, **exocrine pancreatic ducts** can become obstructed, causing **retention of the digestive enzyme trypsin** & "spill-over" into the circulation.
- Detection of this ↑ circulating trypsin is the basis of a **neonatal screening test for CF**. Sampling after 8w risks **false negatives** due to development of exocrine pancreatic insufficiency.

inhibin A 1d 1.5mL

Sent

See inhibin B & FSH.

- **Give day of menstrual cycle in request**.

Female	Pre-menopause (varies with cycle)	5 – 160 ng/L
	Post ..	0 – 3.6
Male	Post-pubertal	0 – 3.6
Sheffield Protein Reference Unit		

Use

- Monitoring histologically diagnosed:
  - **granulosa cell** tumours of the ovary
  - **Sertoli cell** tumours of the testes.

} **inhibin B preferred**

Background

- A 32 kDa dimeric glycoprotein secreted by **granulosa cells** of ovarian follicles & **Sertoli cells** of the testes, which feeds back to the pituitary to **inhibit FSH** secretion. See figure in LH.
- There are **2 types** of inhibin:
- **Inhibin B** is secreted by all ovarian granulosa cells & testicular Sertoli & Leydig cells.
- **Inhibin A** comes from sub-populations of these cell types ie. **not all tumours will secrete it**
- They have a common **α chain** & one of 2 types of β chain: **β A** in inhibin A & **β B** in inhibin B.
- The **placenta** is the main source of inhibin in pregnancy.
- **Menstrual cycle**: low plateau in 1st phase, small mid-cycle peak on a gradual **large rise** & fall in the 2nd phase.
- **Menopause**: similar fall with loss of follicles as inhibin B, but inhibin A lags by about a year.

inhibin B 1d 1.5mL

Sent

See AMH, FSH & inhibin A.

- **Give day of menstrual cycle** with request.

Female	Pre–menopause (varies with cycle)	0 – 341 ng/L
	Post ..	0 – 4
Male	Post–pubertal	25 – 325
Children	Age & sex-related ref. data are sent with results.	
Sheffield Protein Reference Unit		

Use

- Monitoring **granulosa cell** tumours of the ovary & **Sertoli cell** tumours of the testes. Preferred over inhibin A.
- Ix DSDs.

Background

- See inhibin A for details of structure & physiology.
- Inhibin B is **a more sensitive tumour marker than inhibin A**, which sometimes is not secreted.
- But it is **less specific** than inhibin A.
- Levels vary with the **menstrual phase** & **menopause**.
- Inhibin B is the major circulating form in **males**, rather than A.
- **Males:** inhibin B is high at birth, lowest from 1 – 10y & then rises with puberty (Andersen 1998).
- **Females**: similar pattern but the peak at birth is no higher than the postpubertal level (Sehested 2000).
- **Menstrual cycle**: rises & falls in 1st phase, mid cycle peak & then low plateau in 2nd phase: opposite of inhibin A
- **Menopause**: ↓ number of follicles → ↓ inhibin B conc. → ↓ inhibition of FSH secretion → ↑ FSH conc.
- Inhibin B (& AMH) can also be used as a marker of the presence of Sertoli cells (testes) in the Ix of disorders of sexual development (**DSDs**).

insulin 0d 1.5mL

See β hydroxybutyrate, carnitine, CgA, comments, C-peptide, Cr, FFA, glucose & sulphonylurea.

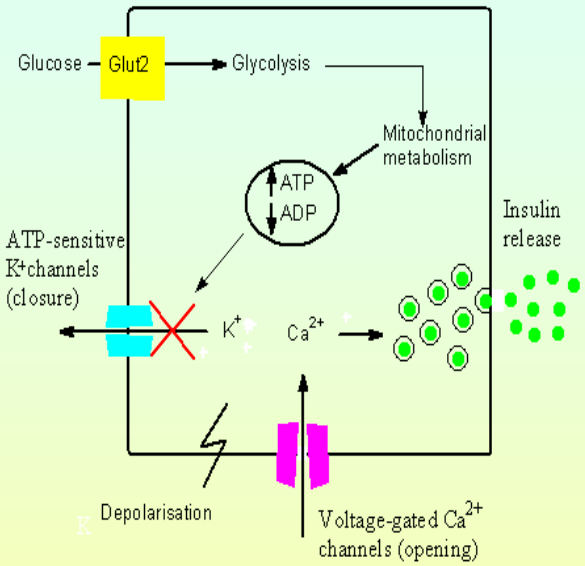
Sent

Interpreted relative to glucose.

Royal Surrey County Hospital

- **MUST deliver to the lab. in minutes**.
- **MUST take blood for glucose** assay by lab.
- **MUST give clinical details.**

Hypoglycaemia is crucial to interpretation	
< 60 y old	hypoglycaemia = ≤ 2.0 mmol/L
> 60 y	.. .. ≤ 2.5 mmol/L



Use

- Ix of **hypoglycaemia**. Must be present because insulin levels are highly variable & can be interpreted robustly *only* when they ought to be suppressed. Blood for glucose assay (grey cap) **MUST** be sent with blood for insulin.
- Ix of exceptional insulin **resistance** eg. in acanthosis nigricans & Donohue syn.
- Ix type of DM, especially in children. Clininical details are essential

Background

- **Beta cell intracellular glucose conc**. parallels that in the interstitial fluid, ie. entry is independent of insulin.
- ↑ glucose conc. leads to ↑ **ATP production** & closure of cell membrane ATP-sensitive potassium channels.
- The ↓ loss of intracellular K → ↓ resting membrane potential → **beta cell depolarisation** → opening of **Ca channels** in the cell membrane → Ca inflow & ↑ **intracellular Ca** conc. → migration of **vesicles of stored insulin** to the cell membrane & discharge of their contents to the exterior.
- Insulin is synthesised as an **86aa precursor**, proinsulin, which is cleaved in the secretory vesicles to form insulin & an equimolar quantity of C-peptide.
- Insulin is a dimer composed of a 30 aa long **B-chain** linked by 2 disulphide bonds to a 21 aa **A-chain**, which contains a third disulphide bond within its sequence..
- Insulin acts via a **tyrosine kinase receptor** of the growth factor family ie. like IGF 1 & 2.
- **Diagnosis of diabetes is little helped by** insulin assay, because levels overlap in health & types 1 & 2. However, with fasting, glucose assay, C-peptide assay & clinical details with the request, the ref. lab. experts can help.
- Fasting insulin levels **add little to the management of insulin resistance in predictable states of resistance eg. obesity**, because of overlap of levels in normal & diagnostic groups due to wide biological & assay variation.

iron 3d See profiles

See acute phase response, CRP, ferritin, folate, iron satn, TIBC, UIBC, & vit B12.

Daily

Age	Female	Male
Neonate	6.4 – 33.0 umol/L	6.4 – 33.0 umol/L
1d – 30d	5.2 – 22.7	5.7 – 20.0
1m – 12m	4.5 – 22.6	4.8 – 19.5
1y – 3y	4.5 – 18.1	5.2 – 16.3
4 – 6y	5.0 – 16.7	4.5 – 20.6
7 – 9y	5.4 – 18.6	4.8 – 17.2
10 – 12y	5.7 – 18.6	5.0 – 20.0
13 – 15y	5.4 – 19.5	4.7 – 19.7
16 – 18y	5.9 – 18.3	4.8 – 24.7
Adult	6.6 – 26.0	10.6 – 28.3

Roche Cobas IRON2 method sheet V6 & Reference ranges for adults & children Roche, 2004.

Use

- Ix of **iron deficiency** (iron assay is used to derive **iron satn.** which is less vulnerable to artefact).
- Screening for **haemochromatosis** (actually, iron satn.).

Background

- Only about **10%** of iron in food is absorbed, but can ↑ up to 2x by:
  - ↑ **dietary citrate** & **vit. C** which promote ferrous (Fe^{2+}) rather than ferric (Fe^{3+}) iron. Fe^{2+} is absorbed better.
  - **iron as haem** (meat)
  - ↑ **need** eg. pregnancy, growth, menses, other blood loss.
- Absorbed in **duodenum** & **proximal jejunum**.
- Transported in plasma as **ferric iron** bound to **transferrin**.
- Serum iron is **only 0.1%** of the total in the body.
- Iron is **a poor index** of iron stores ie.
  - **up to 3 fold variation** day-to-day without apparent reason
  - ↓ with the **acute phase response**.
- Iron saturation is less vulnerable but it too may ↓ with the acute phase response.
- Iron saturation = $\frac{\text{serum iron}}{\text{serum iron + UIBC}} \times 100\ \%$
- Ferritin is the **best marker of iron stores**, but it ↑ with inflammation, which may mask low Fe stores.

Causes of ↓

- chronic Fe deficient diet /blood loss
- diurnal rhythm, ↓ in evening, ↑ in am.
- acute & chronic inflammation eg. bad cold & Rh dis.
- pregnancy if *not* on Fe Px (Fe satn. = N or ↓)
- nephrotic syndrome
- early treatment of pernicious anaemia
- MI

Causes of ↑

- Fe overload eg. haemochromatosis
- acute Fe poisoning
- post-ingestion of Fe Px (peak at 4 – 8h)
- pregnancy if *on* Fe Px (Fe satn = N)
- oestrogens eg. OCP (Fe satn. = N)
- premenstrual (Fe satn. = N)
- pernicious, aplastic & haemolytic anaemia
- acute hepatitis
- vit B6 deficiency

iron saturation 3d See profiles

See CDT, comments, ethanol, ferritin, folate, GGT, iron, **MRI**, TIBC, UIBC & vit B12.

Daily

Women	15 – 50 %
Men	20 – 50 %
Tietz	

Use

- Ix of **iron deficiency** & **overload**.

Background

- Iron satn. is the **primary screening tool for haemochromatosis** & is the measured serum iron conc. expressed as a fraction of the total that can be bound by protein in the patient sample (**transferrin** largely).
  ie. **iron saturation** = $\frac{\text{serum iron}}{\text{serum iron + UIBC}}$ x 100 %
- This test is inexpensive & less vulnerable to interference from the acute phase response than ferritin & serum iron concentrations, which ↑ & ↓ with inflammation, respectively.

Causes of ↓

- chronic Fe def. / blood loss
- pregnancy, no Fe Px (on Px, satn. = N)
- diurnal rhythm, ↓ in evening, ↑ in am.
- chronic illness (eg. infection, Rh, SLE, cancer)
- acute phase response eg. infection, MI

Causes of ↑

- Fe overload
- post-ingestion of iron therapy
- thalassaemia
- cirrhosis
- some anaemias (haemolytic, megaloblastic, aplastic, sideroblastic)
- nephrotic syndrome

K

ketones

ketones (plasma).
See β hydroxybutyrate.

L

lactate (CSF) 1d 0.2mL

Daily

See biotinidase, lactate (venous plasma) & LDH.

Neonate	1.1 - 6.7 mmol/L
3 - 10d	1.1 - 4.4
> 10d	1.1 - 2.8
Adult	1.1 - 2.4
Roche Cobas LACT2 method sheet V4	

Use

• Ix of **neurological disease due to metabolic disorders** which have in common, ↓ ability to complete the oxidation of lactate eg. mitochondrial respiratory chain defects.

Background

• CSF lactate conc. is higher & **independent** of the plasma conc. ie. it can ↑ despite normal plasma lactate.
• This could be due to:
- **naturally high lactate prodution** in the brain due to its high energy need.
- **tissue specifc activity** of its electron transport chain proteins.
- **slow passage across the blood brain barrier** of the strongly ionised lactate anions .

Causes of ↑

1) **Non-specific** response to brain disease:

- ischaemia
- hypoxia
- hypocapnia
- abscesses
- trauma
- CVA
- ↑ ICP
- encephalitis
- intracranial neoplasia
- seizures
- bacterial & fungal meningitis

2) **Inborn Errors of Metabolism** (IEM) which affect the CNS:

- *pyruvate dehydrogenase* deficiency
- mitochondrial respiratory chain defects – may also affect muscle, liver, kidneys, growth etc.
- *biotinidase* deficiency
- nonketotic hyperglycinaemia

NB.

- **CSF lactate may be normal** despite an IEM.
- **Plasma lactate may be normal** despite an IEM & ↑ CSF lactate.

lactate (venous plasma) 3d 2mL

Daily

Neonate	< 2.9 mmol/L
Adult or Child	0.5 - 2.2
Roche Cobas LACT2 method sheet V4	

See anion gap, bicarbonate, ethylene glycol, lactate (CSF), LDH & salicylate.

- **Blood must be free flowing**
- **Take to lab. immediately**

Background

- **Glycolysis**, the anaerobic catabolism of glucose to pyruvate with the production of ATP, ceases in the absence of the coenzyme NAD (unreduced nicotinamide adenine dinucleotide).
- The Tricarboxylic Acid Cycle needs NAD too, but it does not function under anaerobic conditions anyway.
- **Pyruvate can be reduced to lactate** (catalysed by LDH).

- This converts NADH (reduced NAD) produced by glycolysis, back to NAD.
- The NAD produced enables further glycolysis to yield more ATP, albeit only a fraction of what the TCA cycle could make if it was not disabled by hypoxia.

- The **reaction can be reversed** to produce pyruvate from lactate**:**

- in the **liver** the pyruvate can be used for **gluconeogenesis** (the Cori cycle).
- in **other tissues**, pyruvate enters the TCA cycle for **energy** production .

Causes of ↑ Lactic acidosis can be divided into **2** types**:**

Type A caused by **tissue hypoxia**, the commonest type eg.

- ↓ perfusion
- hypoxaemia
- severe anaemia
- CO poisoning.

Type B, not primarily due to hypoxia. There are **3** overlapping categories of cause

1. Underlying disease eg.

- sepsis
- liver failure
- renal failure
- cancer eg. NH lymphoma
- DKA
- muscle activity eg. malignant hyperthermia

2. Drugs & intoxicants eg.

- ethanol
- methanol
- cyanide
- ethylene glycol (anti-freeze)
- salicylate
- TPN
- metformin
- ecstasy
- cocaine

3. Inborn errors of metabolism eg.

- *glucose-6-phosphatase* def. (GSD I)
- *fructose-1,6-diphosphatase* deficiency
- mitochondrial disease eg. MELAS
- *pyruvate dehydrogenase* deficiency

lactate dehydrogenase (LDH) 3d 1.5mL

Daily

See BJP, β_2M, cTnT, lactate (CSF) & lactate (venous plasma),

Age	LDH U/L
4 – 20d	225 – 600
2 – 15y	120 - 300
Adult female	135 - 214
.. male	135 - 225
Roche Cobas LDHI2 method sheet V4	

Use

- a non-specific **tumour marker**.

Background

- **Abundant in all cells** eg. rbc & wbc.
- A **tetramer** of H & M protein chains.

- Heart LDH has 4 H.
- Liver & striated muscle LDH have 4 M ones.
- LDH in other tissues contains a mixture.

- **Electrophoresis** distinguishes the types of LDH & was used previously to detect myocardial LDH as evidence of MI. Cardiac troponin T assay has replaced this use, because cTnT is more sensitive & is truly cardio-specific.
- LDH catalyses the **reduction of pyruvate** to **lactate**. This regenerates NAD from the reduced form (NADH). Without NAD, glycolysis would stop & ATP production would fall even further under anaerobic conditions.
- **LDH also catalyses the reverse** ie. oxidation of lactate to pyruvate.
- Lactate, particularly from muscle, circulates to the liver where LDH crucially converts it to pyruvate to feed gluconeogenesis for export to peripheral tissues (the **Cori cycle**).
- Despite poor specificity, LDH is still used as a **marker** of neoplastic cell mass in the absence of alternatives eg.

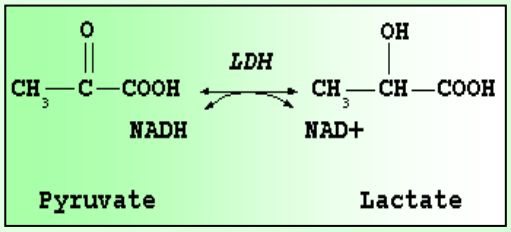
- lymphoma
- leukaemia
- seminomatous germ cell carcinoma.

LDH assay is inexpensive & easily repeated to overcome imprecision.

- **CSF LDH** is ↑ by bacterial & fungal meningitis, SAH & intracranial neoplasia. However, LDH assay **adds minimally** to much better tests eg. microbiology, bilirubin spectrophotometry & brain imaging.

Causes of ↑

- Haemolysis
- **any** tissue injury eg. of liver, skeletal muscle or cardiac muscle
- ↑ wbc mass eg. lymphoma, leukaemia.



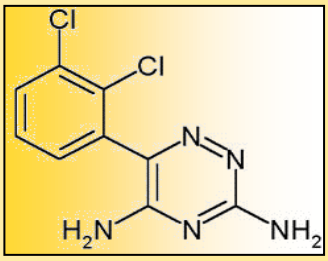
lamotrigine 3d 2mL

Sent

0.0 – 15.0 mg/L
St Helier Hospital

See carbamazepine, phenobarbitone, phenytoin, TDM, valproate & zonisamide.

- **Pre-dose sample.**



Use

Not for routine monitoring.

- Ix - toxicity
  - non-compliance
  - loss of clinical effect or difficulty in establishing it, especially with co-Px

Background

- Lamotrigine is for **epilepsy, bipolar disorder** & **neuropathic pain**.
- The case for therapeutic drug monitoring (TDM) is weak ie. dose predicts effect.
- The mechanism of action may be inhibition of the excitatory neurotransmitter glutamate & stabilization of neurones
- 90% is **metabolised** & glucuronidated in the **liver,** then excreted by kidney.
- **10%** is **excreted unchanged** in urine.

Causes of ↑

- co-Px valproate (lamotrigine can ↓ valproate levels) • hepatic impairment • ↓ GFR.

Causes of ↓

- co-Px carbamazepine (lamotrigine can ↑ the level of the carbamazepine epoxide metabolite, causing toxicity)
- co-Px phenobarbitone & phenytoin (potent hepatic enzyme inducers).
- oestrogens eg. OCP (level may increase in the "pill-free time").

LDL (low density lipoprotein) cholesterol 3d See profiles

Daily

See cholesterol (total), HDL, **MRI**, cholesterol:HDL ratio & Tg.

- **Ideally fasted.**
- **If Tg is > 4.5 mmol/L, LDL cannot be calculated.**

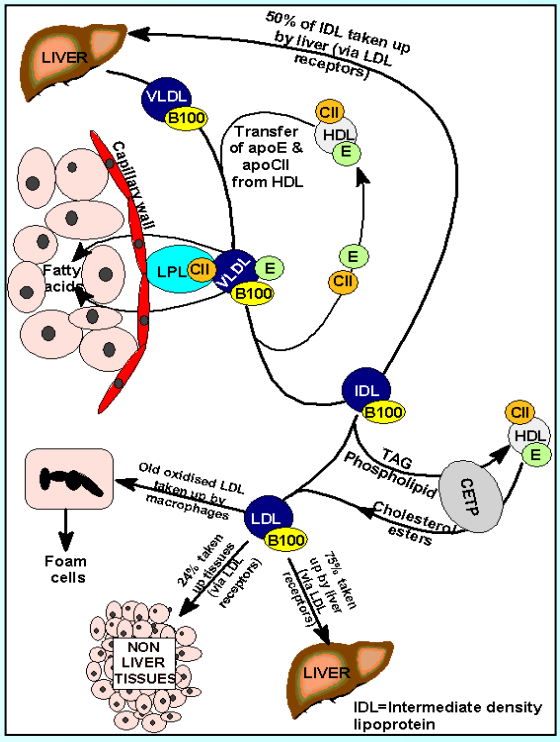
Desirable < 2.0 mmol/L
J.B.S. 2005

Use • Ix of CVD risk & response to Px.

Background

- See cholesterol (total) for LDL **target** levels.
- See HDL for more lipid biology.
- **Lipids are insoluble in water**. For transport in plasma this is overcome in ways similar to cell membranes:

- Lipid molecules **aggregate** in the hydrophobic interior of lipoprotein particles.
- On the surface, **amphipathic** substances (hydrophobic & hydrophilic regions in the same molecule) eg. phospholipids & cholesterol esters, bridge the otherwise incompatible aqueous & hydrophobic environments
- Proteins on the surface (**apoproteins**) determine interactions with specific enzymes & tissues.



- LDL has a round-about genesis, like HDL **:**

1 VLDL (very low density lipoprotein) secreted by liver, has:

- high triglyceride (Tg)
- 20% esterified cholesterol
- apoproteins **B100, C** & **E.**

ApoC activates **lipoprotein lipase** (LPL) in adipose tissue to remove Tg as fatty acids. This ↑ the proportion of cholesterol remaining & forms **IDL** (intermediate density lipoprotein).

2 IDL interacts with **HDL** to further ↑ its **esterified cholesterol** & apoC content.

3 IDL to LDL conversion is completed by removal of more Tg, mostly by **hepatic triglyceride lipase** in the liver vascular endothelium, but a little by LPL.

- **B100** is the characteristic apoprotein of LDL & it is the ligand for the **LDL & IDL receptor**.

- The **liver removes 75%** of LDL cholesterol by this receptor-mediated route & recycles or excretes it as **bile acids**.
- **25%** of LDL cholesterol is taken up by other tissues eg. endothelium.

Causes of ↑

- primary hyperlipoproteinaemia IIa & IIb
- hypothroidism
- nephrotic syn.
- CRF
- cholestasis
- anorexia nervosa
- metabolic syn.
- pregnancy
- porphyria
- Cushing's syn.

Causes of ↓

- hypo & a-β-lipoproteinaemia
- α-lipoprotein def (Tangier dis.)
- LCAT def.
- Apo C-II deficiency
- hyperthyroidism
- liver failure
- myeloma
- cancer
- acute phase response

lead (inorganic Pb exposure) 3d 2mL

See Hg inorganic, lead (organic) & MRI.

Sent

Exposure	Whole blood Pb
• Environmental	< 0.5 umol/L
• Occupational	< 1.4
Royal Surrey County Hospital	

- **A service cannot be provided for occupational health monitoring outside SASH NHS Trust.**

Use & Background

- The NHS provides lead analyses only when lead intoxication might be the cause of a patient's symptoms & signs**:**
  - abdominal pain
  - anaemia
  - blue line on gums
  - fatigue
  - CRF
  - X ray signs
  - weakness
  - peripheral neuropathy
  - basophilic stippling of RBCs
- **Children** suffer severe effects more commonly than adults eg. encephalopathy & death.
- The effects of **organic lead** intoxication are more neurological & are better studied with **urine** samples.

lead (organic Pb exposure) 3d 24h urine

See Hg organic & lead (inorganic).

Sent

< 100 nmol/24h
Royal Surrey County Hospital

- **Procedure**: Urinate in the toilet & note the time (can be any time). Collect all urine for the next 24h. The last one to collect is whatever can be passed at the noted-time next day.
- **A service cannot be provided for occupational health monitoring outside SASH NHS Trust.**

levetiracetam (Keppra) 3d 1.5mL

See therapeutic drug monitoring.

- **Sample pre-dose**

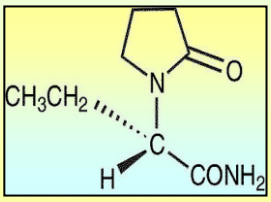
Sent

12 – 46 mg/L
Chalfont Centre for Epilepsy

Use

- **Routine monitoring of serum levels is unnecessary**
- Ix suspected toxicity, non-compliance & loss of clinical effect or difficulty in establishing it.

Background



- Used to treat epilepsy & neuropathic pain.
- Mechanism of action is unclear.
- Linear pharmacokinetics ie. the clinical effects & toxicity are predictable from the dose
- Clearance:

- **66%** excreted **unchanged in urine**
- **24% hydrolysed** to inactive products.

- **↓ GFR ↓ elimination** & **↑ serum level**.
- Levetiracetam has **little impact on the elimination of other drugs**, not being metabolised by cytochrome P450.

LH (luteinising hormone) 3d See profiles

See AMH, FSH, hCG, inhibin A, LHRH test, oestradiol, prolactin, progesterone, testo & TSH

Daily

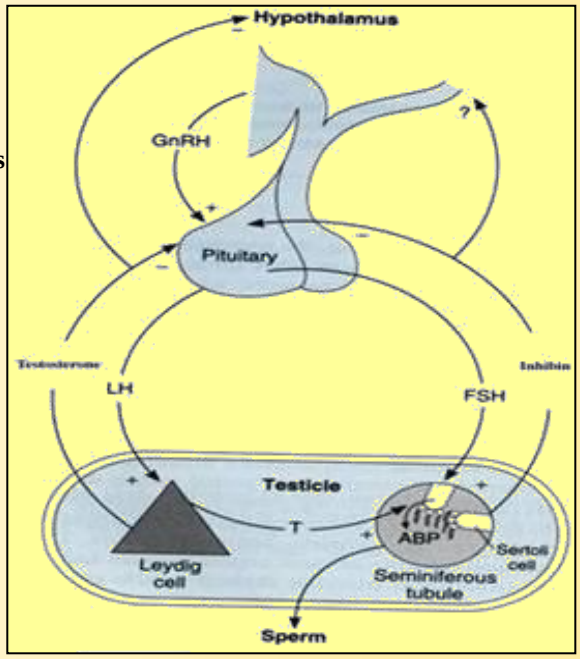
		LH (IU/L)
Women	Follicular phase	2.4 - 12.6
	Mid-cycle peak	14.0 - 95.6
	Luteal phase	1.0 - 11.4
	Menopause	7.7 - 58.5
Men		1.7 - 8.6
Roche Cobas LH method sheet V19 (5th - 95th cent.)		

Background

- A **heterodimeric** glycoprotein with the same **α-chain** as FSH, TSH & hCG but a unique **β-chain**.
- Secretion of LH is **pulsatile** by **gonadotropes** of the **anterior pituitary** in response to pulsatile gonadotropin releasing hormone (GnRH/LHRH) in the portal blood stream from hypothalamic neurones.
- **WOMEN**: in concert with FSH, LH regulates:

- **follicle growth** & maturation
- **ovulation**
- formation of the **corpus luteum**
- synthesis of **oestradiol** & **progesterone** (see FSH).

- **MEN**: LH stimulates **testosterone** secretion by **Leydig cells** of the testis.
- **BOTH** sexes: LH (& GnRH) release is **inhibited by** testosterone & oestradiol. Even in men, it is actually oestradiol made from testosterone by locally expressed **aromatase**, which mediates most **negative feedback**.
- **Women** are more complicated than men & there is additional transient **positive feedback** which leads to the **mid-cycle LH surge** & ovulation.



Causes of ↑

- **Primary gonadal failure** eg. menopause, Kleinfelter's & Turner's syndromes (↑ is less than shown by FSH).
- **Polycystic ovary syndrome** (PCOS).

Causes of ↓

- **Secondary gonadal failure** (hypogonadotropic hypogonadism) eg. Kallmann's syn., hypothalamic or pituitary deficiency, physiological response to ↓ Wt. or debilitation.
- pregnancy
- exogenous sex steroids (including body-building drugs)
- LHRH analogues
- pre-puberty

lithium 3d 1.5mL

Daily

0.4 – 0.8 mmol/L
Roche Cobas LI method sheet V9

See Ca, comments, drugs & TFTs, eGFR, FT4, TSH & therapeutic drug monitoring.

- **Pre-dose sample.**
- **Monitor TFT** & **U+E** too.

Use • Optimisation & monitoring Li therapy**.**

- **NB.** The upper reference limit depends on the **psychiatric need** ie. to control acute states, levels up to 1.2 mmol/L can be an acceptible **risk/benefit balance**.
- Test after 4-7 d of treatment, then weekly until dosage has remained constant for 4 weeks, then 3 monthly on stabilised regimes.
- Check level if **formulation** or **fluid intake changed** or if **interacting drugs** are added/withdrawn.
- 100% renal clearance ie. beware ↓ GFR
- Test serum Li up to 4 hourly in overdose situations requiring intensive therapy. BNF (2012).

M

macroprolactin 1d 1.5mL

in-house, 1–2 d

See FSH, LH, **MRI**, oestradiol, prolactin & TRH test.

	Ref. ranges for PEG treated serum (mU/L)	
	Before PEG	**After** PEG
Female	102 – 496	75 – 381
Male	86 – 324	63 – 245
	Roche Cobas Prolactin II method sheet V6 (2.5^{th} – 97.5^{th} centiles)	*Beltran et al 2008 (2.5^{th} – 97.5^{th} centiles)*

Use

- Ix of hyperprolactinaemia especially if inconsistent with clinical details, tests, imaging or therapy.

Test

- Prolactin is assayed in serum before & after treatment with polyethylene glycol (PEG), which precipitates high MWt. forms more than monomeric, bioactive prolactin.

Proportion of prolactin left after PEG, if ↑ is due to: *Schlechte 2002*		
↑ monomer	↑ macro prol.	↑ both
≥ 60 %	**≤ 40%**	**≈ 40–60 %**

Background

Summary of prolactin forms & properties:

Forms of prolactin	**M Wt** kDa	**Proportion** %	**Biological activity**
Monomer	23	30 - 85	active
Dimer *big prolactin*	≈ 50	9 - 23	inactive
Tetramer	≈ 100	0.5 - 5.0	inactive
IgG aggregate *big big prol.*	≈ 150	2 – 55 mean 13	inactive
Roche Cobas Prolactin II method sheet V6 & Beltran et al 2008			

- Prolactin is a **198** a.a. **glycoprotein** secreted by the **lactotropes** of the anterior pituitary**.**
- Circulating prolactin is a mixture of forms, each of which can have variable glycation too:
- **Bioactivity** lies in the monomeric form which is normally the most abundant.
- **Bioavailability** of the ↑ MWt forms is ↓ because they escape capillaries & reach the site of action less.
- **Macroprolactin** (big big prolactin) = **aggregated monomer & IgG**, 10-20% of cases of hyperprolactinaemia.
- It is **harmless**, but the ↑ prolactin results created, can cause diagnostic confusion & needless Ix.

CAUTION. The above is simplistic:

- Macroprolactin actually has **normal bioactivity** *in vitro*, but its low bioavailability negates it Valette-Kasic 2002.
- ↑ total prolactin due to macroprolactinaemia in asymptomatic people who are negative on all Ix, is easily dismissed as artefact. But what of patients who are the same, but symptomatic for hyperprolactinaemia eg. infertility, menstrual irregularity & galactorrhoea? Valette-Kasic 2002
- ↑ monomeric prolactin *and* macroprolactinaemia may **co-exist** eg. prolactinoma. Fine if visible on imaging, but what if it is not? Assays which examine the **ratio** of macro to monomeric prolactin can fail to reveal ↑ of the latter because it suffers up to 40 % loss in the reaction. See above.
- **Ref. ranges** (see above) for sera of healthy people which has been subjected to the same PEG treatment can help to reveal ↑ monomeric prolactin too. Beltran et al 2008.
- **Macroprolactin** constitutes **up to 55%** of total prolactin even in health. Beltran et al 2008.

magnesium, Mg (serum) 3d 1.5mL

Daily

See Ca, comments, digoxin, Mg (urine) & PTH.

Neonate	0.62 – 0.91 mmol/L
5m – 6y	0.70 – 0.95
6y – 12y	0.70 – 0.86
12y – 20y	0.70 – 0.91
20y – 60y	0.66 – 1.07
60y – 90y	0.66 – 0.99
> 90y	0.70 – 0.95
Roche Cobas MG2 method sheet V7	

Background

- Mg is a cofactor for many **enzymes** eg. ATP-dependent ones.
- **Mg is mostly intracellular** ie. serum levels may be normal despite ↓ total body content.
- 70% of Mg is stored in **bone** & 35% of plasma Mg is **protein bound**
- The physiology of Mg regulation is unclear, but:

- **aldosterone** ↑ urinary excretion
- **PTH** effects are like those it has on serum Ca.

Clinical effects of Mg levels.	
Onset of symptoms of depletion	0.50 mmol/L
Tenany	0.15 - 0.50
Cardiac-conduction impairement	2.50 - 5.00
Loss of tendon-reflexes	5.00 - 6.50
Roche Cobas MG2 method sheet V7	

Causes of ↓ eg.:

- protein-calorie malnutrition
- malabsorption
- steatorrhoea
- chronic diarrhoea
- dietary factors (phytate, PO_4 & fatty acids ↓ absorption)
- renal tubular disease eg. due to hypercalcaemia
- physiologically in the 2nd & 3rd trimesters of pregnancy
- diuretics
- alcoholism
- hyperaldosteronism
- DKA, especially with treatment

Effects: begin when serum **Mg is ≤ 0.50 mmol/L**.

- weakness
- irritability
- ecg changes
- coronary artery spasm.
- tetany
- delerium
- fitting
- ↑ sensitivity to digoxin.
- ↓ **serum Ca** & **K** which resist replacement until ↓ Mg is corrected (Mg enables PTH secretion & response).

Causes of ↑ (less common than ↓) eg.:

- ARF
- CRF
- dehydration
- trauma/burns
- Addison's disease
- uncontrolled diabetes
- ↑ intake eg. ivi & antacids
- Li
- hypothyroidism

Effects: Begin when serum **Mg > 2.0 mmol/L**, exaggerated by ↓ Ca or ↑ K

- ↓ tendon reflexes
- muscle weakness
- ↓ BP
- bradycardia
- potentiates cardiac effect of ↑ serum K
- ileus
- hypocalcaemia

magnesium, Mg (urine) 3d 24h urine
See Mg (serum).

Daily

3.0 – 5.0 mmol//24h
Roche Cobas MG2 method sheet V7

- **Procedure**: Urinate in the toilet & note the time (can be any time). Collect all urine for the next 24h. The last one to collect is whatever can be passed at the noted-time next day.

mercury (organic Hg exposure) 3d 5mL
See mercury (inorganic) exposure & Pb (organic).

Sent

< 20 nmol/L
Royal Surrey County Hospital

- As for lead, **all workers at risk should be part of an occupational health scheme**. The **NHS will not** process specimens from these people, only samples from patients with illness possibly due to Hg toxicity.

Background

- Organic Hg is found naturally in many **long-lived** & **large fish** eg. shark, tuna & swordfish.
- **Smaller fish** with shorter lifespans, have **lower level**s.
- The advice to eat fish 2x a week for CVD prevention remains valid, but pregnant women should eat fish with a low mercury content.
- Organic Hg, being **lipophilic**, is stored in fat & can take a year to be excreted after cessation of intake.

.

mercury (inorganic Hg exposure) 3d 20mL
See mercury (organic) exposure & Pb inorganic.

24h urine

Sent

< 5.5 nmol/mmol creat.
Royal Surrey County Hospital

< 50 nmol/24h
Royal Surrey County Hospital

- **Procedure**: Urinate in the toilet & note the time (can be any time). Collect all urine for the next 24h. The last one to collect is whatever can be passed at the noted time next day
- **Amalgam dental fillings** do not significantly expose owners to risk or detectably ↑ mercury excretion.
- As with lead, **workers at risk should be part of an occupational health scheme**. The **NHS cannot** process specimens from these people, only samples from patients with illness possibly due to Hg toxicity.

metadrenaline 3d acidified 24h urine
(metanephrine). See VMA

- **Plain bottle.**
- **Acid is added on its return to the lab.**

Sent

< 1.2 umol/24h
St Helier Hospital

Children only, 20mL urine

Methaemoglobin 0d 3mL

or a **heparinised blood gas syringe.**

See blood gases.

< 3.0 %
IL GEM 4000 reference guide

Use

- Ix of methaemoglobinaemia

Background

- 1 Hb molecule has **4 haem** groups, each can carry 1 molecule of oxygen.
- Each haem has an Fe^{2+} (**ferrous**) ion at its centre, even in oxyHb.
- In MetHb, the **oxidized haem** (ferric, Fe^{3+}) cannot carry oxygen & causes reduced haem groups (ferrous, Fe^{2+}) to have ↑ affinity for oxygen ie. impaired release to tissues (left shift of the O_2 dissociation curve).
- Hb with Fe^{3+} (MetHb) is normally at low levels (**< 3.0 %**) because it is swiftly reduced.
- **Cyanosis** is visible with 10 – 20 g/L of MetHb.

Causes of ↑ (acquired)

- Drugs eg. chloroquine, dapsone, isoniazid, lignocaine, metoclopramide, nitrites, smoking
- Poisoning with oxidising agents eg. chlorate, nitrite

Causes of ↑ (hereditary)

- Deficient activity of ***NADH – MetHb reductase*** (cytochrome b_5 reductase, diaphorase): recessive.
- Variant Hb – haemoglobin M: dominant.

3-methoxytyramine 3d acidified 24h urine

(dopamine metabolite). See VMA.

- **Plain bottle.**
- **Acid is added on return to the lab.**

Sent

< 2.5 umol/24h
St Helier Hospital

Children only, 20mL urine

Background

- **3-methoxytyramine** is a **dopamine** metabolite which ↑ with ↑ dopamine secretion by non-renal disease eg. neuroblastoma ie. it helps in the **distinction of renal from non-renal** causes of ↑ urine dopamine excretion.

methylmalonic acid (MMA) 0d 5 mL Sent

See folate (serum), homocystine, Igs & vit. B12.

- **Clinical details needed.**

Serum MMA	INTERPRETATION
< 0.29 umol/L	Not indicative of vit. B12 deficiency
0.29 – 0.70	Suggestive .. ..
> 0.70	Consistent with overt .. .. ..
Royal Sussex University Hospital, Brighton	

Use

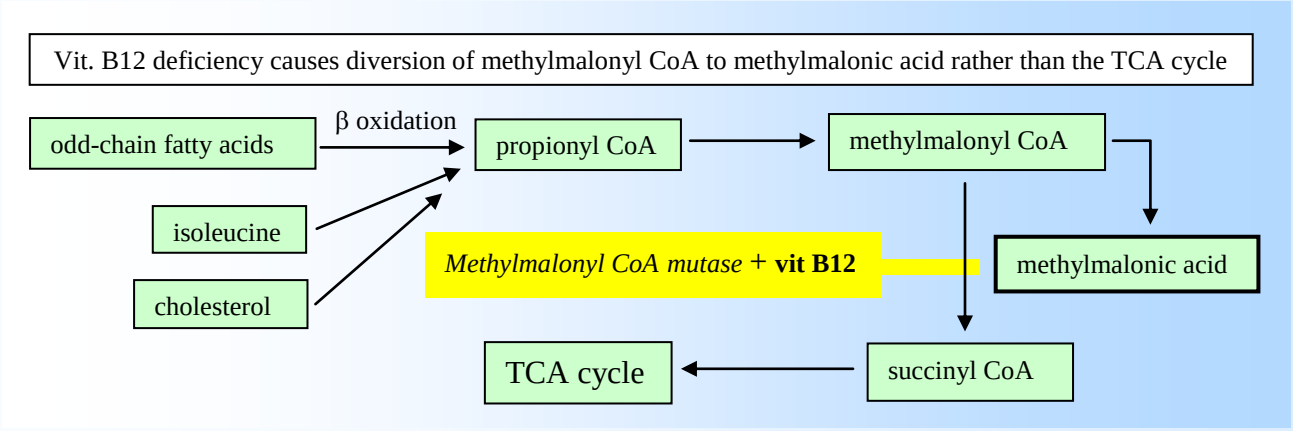
- Ix of unclear vit. **B12 deficiency**.

Plasma homocystine	MMA	INTERPRETATION
↑	↑	Compatible with B12 def.
normal	normal	B12 deficiency unlikely.
↑	normal	Folate deficiency possible
Interpretation if plasma homocystine available too. Ditto.		

Background

- B12 is a cofactor for ***methyl-malonyl CoA mutase*.**
- MMA is a **marker of vit. B12 function:** B12 def. causes ↓ enzyme activity & accumulation of its substrate, methylmalonyl CoA, which is alternatively hydrolysed to methylmalonic acid (MMA). See DIAGRAM below.
- 60% of cases of **methylmalonic aciduria** (recessive, 1:40k births) involve this enzyme.
- **Haematinic testing:**

- serum folate & vit. B12: 1st line tests & *sufficient for nearly all cases.*
- plasma homocystine } 2nd .. .. if serum folate &/or vit. B12 levels do not adequately explain clinical
- serum MMA } evidence of deficiency.



6-methylmercaptopurine nucleotides (MMPN) 3d 1mL

Sent

See DYPD, thioguanine nucleotides & TPMT.

- **Sample pre-dose.**

< 5700 pmol/8x10^8 rbcs
City Hospital, Birmingham

Use

- Detection of toxic levels in people on azathioprine & related drugs.

Background

- The purine analogue **azathioprine** (AZA) is used to restrain the immune response in transplantation & to treat diseases such as **inflammatory bowel disease** (IBD).
- **AZA** & **mercaptopurine** are metabolised to **thiogunine nucleotide** (TGN), the main product & effector of the therapeutic & toxic effects in most people (see metabolic DIAGRAM).
- Thus, TGN levels are used for **monitoring efficacy & toxicity**.
- However, some patients preferentially metabolise AZA to **methylmercaptopurine ribonucleotides** (MMPN).
- **MMPN is active** & inhibits purine synthesis.
- Extreme levels can cause **hepatic failure** & **myelotoxicity**.
- **If only TGN is assayed**, the AZA dose may be ↑ in an effort to remedy the low TGN levels seen in these patients. The result can be **toxicity from high levels of MMPN** instead.

mycophenolate 3d separated from cells 3mL

(mycophenolic acid)

Sent

See therapeutic drug monitoring.

- **Pre-dose sample**

Depends on the use
King's College Hospital

Use

- Optimising mycophenolate drug therapy.

Background

- An **immunosupressant**, originally from a fungus, used to control rejection of transplanted organs eg. liver & increasingly, in the treatment of psoriasis & immunologically mediated diseases eg. SLE.
- **2 forms**:

- **mycophenolate mofetil,** the original drug, is converted in the liver to the active substance **mycophenolic acid**.
- **sodium mycophenolate** a salt of mycophenolic acid.

- Mycophenolate inhibits ***inosine monophosphate (IMP) dehydrogenase***, the rate limiting enzyme in the synthesis of purines (adenine & guanine) in lymphocytes.
- Often **combined with other immunosupressants** acting at other sites eg. calcineurin inhibitors (cyclosporin & tacrolimus) & corticosteroids (prednisolone).
- Eliminated by **hepatic metabolism** with 95% of a dose ending up in urine.

N

neuron specific enolase (NSE) 3d 3mL

Sent

≤ 12.5 ug/L
Sheffield Protein Reference Unit

Use

- A marker of tumour therapeutic-response & recurrence.
- **Not for diagnostic use.**

Background

- Enolase is a **glycolytic enzyme** which catalyses the conversion of 2-phosphoglycerate to phosphoenolpyruvate.
- It is a **dimer** of **α, β** or **γ peptide subunits** which combine to give distinct **tissue specific forms:**

	TISSUE EXPRESSION
α α	Glia & most tissues
γ γ (NSE)	**Neurons, chromaffin cells & neuroendocrine cells**
β β	A special muscle-form
α γ	Low level in brain & neuroendocrine tissue. Intermediate properties

Causes of ↑

- neuroblastoma
- SCC lung
- melanoma
- hypernephroma.
- pancreatic islet-carcinoma
- other neuroendocrine tumours

neurotensin 0d 6mL + **aprotinin** Contact lab.

See gut hormone profile.

Sent

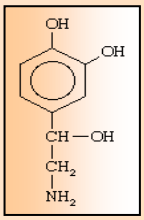
< 100 pmol/L
Charing Cross Hospital

noradrenaline 3d acidified 24h urine

See VMA.

- **Plain bottle**
- **Acid is added on return to the lab.**

Sent



< 500 nmol/24h
St Helier Hospital

Children only, 20mL urine

normetadrenaline 3d acidified 24h urine

(normetanephrine). See VMA.

- **Plain bottle**
- **Acid is added on its return to the lab.**

Sent

< 3.3 umol/24h
St Helier Hospital

Children only, 20mL urine

O

occult blood (faecal) **x 3 Hema-Screen cards.**
See faecal occult blood 1 for each bowel movement

Daily **negative**

- To get cards, email: PathStores@sash.nhs.uk
- **Fresh stool is unacceptible:** degrades too fast
- **Routine F.O.B. testing ceased 1.6.2011**

Use

- **Very limited** outside of the NHS Bowel Cancer Screening Programme. NICE CG27.
- May be useful in symptomatic patients **unsuited to referral & endoscopy** eg. due to psychiatric difficulties.
- Referral to a g.i. specialist should be based on **Hb, gender, age** & **clinical details**. NICE CG27.

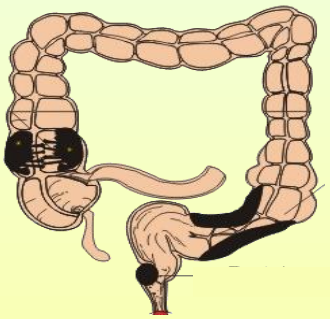
Background

- The **NHS Bowel Cancer Screening Programme** is the **only indication** for FOB testing in **asymptomatic** people. Independent of GP & 2ndy care, test kits are posted every 2y directly to 60 – 75y olds.
- FOB is **NOT recommended** for:

- Ix **symptomatic** possible **g.i. cancer** - performance is poor ie. too many **false – ves** & **false + ves**. NICE CG27
- Ix iron deficiency **anaemia** – BSG 2006

- The FOB test detects the **oxidising property of haem** in Hb. ie. **false + ve** results can be caused by **oxidising agents** eg. dietary peroxidase & **false – ves** by **reducing agents** eg. dietary vitamin C.
- **Haem decays rapidly in wet faeces**, causing false – ves.
- **NB.** Immunoassays of Hb are affected by degradation in wet samples too, albeit less than haem systems..
- Dried Hb on a card is stable for 1 – 2 weeks.

Minimise false + ve results by:



- **EATING a high plant fibre diet:**
  - plenty of raw & cooked veg. eg. lettuce, corn, spinach
  - plenty of fruit eg. plums, prunes, grapes, apples
  - moderate amounts of bran, cereal, popcorn, wholemeal bread, peanuts
- **AVOID THESE FOODS during & for 3 days before collection:**
  - red-meat that is not "well-done" (beef, lamb)
    (can eat fish & well-cooked turkey, chicken & pork)
  - horseradish
  - turnip
  - radish
  - parsnips
  - cauliflower
  - broccoli
  - melon

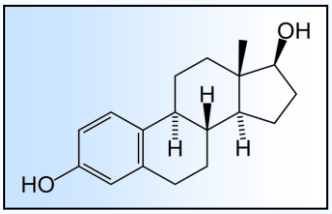
  } (contain peroxidase, false + ve possible)

  - >250mg vit. C/day (reducing effect counters oxidising mechanism of the test, false – ve possible)
- **AVOID THESE DRUGS which cause gut bleeding** eg.
  - aspirin
  - iron supplements
- **AVOID COLLECTION DURING:**
  - menstruation or just before or just after
  - bleeding piles
  - dental work
  - diarrhoea or constipation
  - haematuria
  - consumption of iron supplements
  - intake of drugs which cause g.i. bleeding eg.aspirin, corticosteroids.

oestradiol 2d See profiles

Daily

See AMH, FSH, hCG, LH, LHRH test, MRI, progesterone, prolactin & testosterone.



Women	Follicular phase	46 - 607 pmol/L
	Mid cycle peak	315 - 1828
	Luteal phase	161 - 774
	Postmenopause	≤ 201
	Pregnancy 1st trimester	789 - 15781
Children	girls 1 - 10y	22 - 99
	boys 1 - 10y	18 - 73
Men		28 - 156
Roche Cobas Estradiol II method sheet V3 (5th – 95th centiles)		

Background

• **WOMEN**:

- 95% of oestradiol is secreted by the **granulosa** cells of ovarian follicles, which convert (**aromatise**) **testosterone** received from the surrounding **theca cell** layer.
- 5% of oestradiol is secreted by the **adrenal** cortex & **adipose** tissue where the enzyme **aromatase** converts circulating androgens eg. androstenedione & DHEA.
- The massive oestadiol levels of **pregnancy** arise from **placental synthesis** (suppresses FSH & LH secretion).
- In the **perimenopause** ie. the time between full reproductive function & menopause, highly variable serum oestradiol levels may be seen, which are apparently discordant with FSH & LH levels.

• **MEN**:

- Most oestradiol comes from conversion of circulating testosterone & androstenedione by the enzyme ***aromatase* in adipose tissue**.
- Lesser amounts are secreted by the **testis** & **adrenal cortex**.

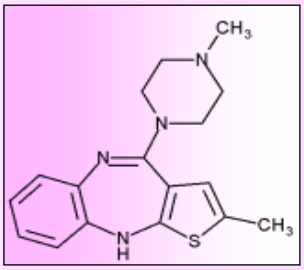
olanzapine 0d 3mL

Sent

20 – 40 ug/L
Kings College Hospital

See clozapine & therapeutic drug monitoring.

• **Pre-dose sample.**
• **Routine drug monitoring is unnecessary**
• **Monitor LFT & FBC** instead for drug side effects



Use

• **Ix of:**

- suspected toxicity or non-compliance
- failure to establish or maintain therapeutic effect.

Background

• an **atypical antipsychotic**
• structurally **similar to clozapine**.
• for treatment of schizophrenia.

oligosaccharides 1d 5mL fresh urine. (sugar chromatography)

or faeces

Sent

See reducing substances (faeces), reducing substances (urine) & sugar chromatography.

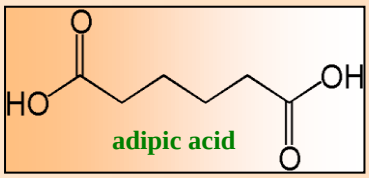
• **Give details of Px:** some drugs interfere.

Use • Ix of the cause of a + ve result for reducing substances in urine or faeces.

organic acids 1d 5mL fresh urine

Sent

Assays + interpretation
Great Ormond Street Hospital



See amino acids, ammonia, β-hydroxybutyrate, carnitine, GAGs, orotic acid, VLCFA & wbc enzymes.

- **Samples taken during clinical episodes are especially useful.**

Use

- Screening for Inborn Errors of Metabolism in children

Background

- Organic acids are **carboxylic acids** with or without non-amino functional groups eg. hydroxyl & keto. Amino acids are not included in this assay, although they may resemble organic acids structurally.
- There is a long list of **inborn errors of metabolism** (IEM) affecting eg.

- carbohydrate catabolism
- amino acid catabolism
- the urea cycle
- organic & fatty acid oxidation

which **alter the levels of urinary organic acids** eg. ↑ adipate & suberate in β-oxidation defects.

- Assay of urine organic acids is **a valuable screen for IEM**, especially in combination with assay of amino acids in serum or plasma,
- **Urine is best**, because the kidneys efficiently clear organic acids from the circulation.

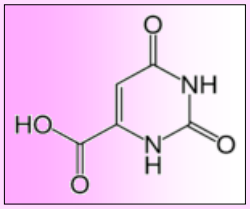
orotic acid 1d 10mL fresh urine

Sent

Refs & comment sent with result
Great Ormond Street Hospital

See ammonia, amino acids & organic acids.

- Orotic acid & organic acids **can use the same sample.**
- Ideally **non-fasting**, on a **normal protein intake** & **during a clinical episode**

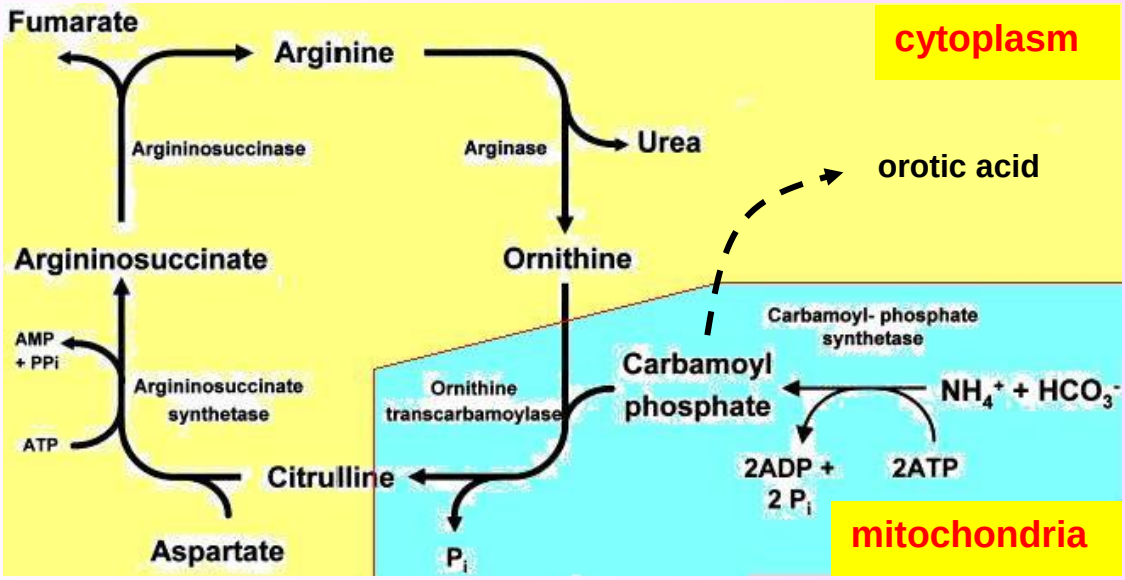


Use

- Ix of urea cycle disorders.

Background

- Orotic acid is a **pyrimidine** & a precursor to the more familiar ones, **thymine & cytosine**, hence its alternative name, pyrimidine carboxylic acid.
- **Urea cycle** enzyme defects (see ammonia) located ***downstream* of carbamoyl phosphate** synthesis eg. *ornithine transcarbamoylase* deficiency, cause **hyperammonaemia** & accumulation of carbamoyl phosphate which is shunted into pyrimidine synthesis ie. **↑ orotic acid synthesis & excretion.**
- Enzyme defects **upstream** of carbamoyl phosphate eg. *carbamoyl phosphate synthetase*, also cause **hyperammonaemia**, but **NOT ↑ orotic acid** synthesis & excretion.



osmolality (serum) 3d 1.5mL

Daily

See comments, ethanol, ethylene glycol, Na (serum), Na (urine conc) & osmo (urine).

Neonates	> 266 mmol/kg
Adults < 60y & **Children**	275 – 295
Adults > 60y	280 – 301
Tietz	

Use

- Ix of **intoxication** eg. ethanol, ethylene glycol.
- Monitoring during a **water deprivation test**.
- **Little value as a screen** for SIADH & DI or Ix hypo & hypernatraemia. See urine osmo. & Na conc.

Background

- Spot serum osmo. **rarely adds anything new**, because the major contributors to serum osmolality (Na, K, urea & glucose) are usually known already.
- For **Ix hypo & hypernatraemia**, it is usually more productive to measure urine osmolality & urine sodium conc.
- Hyponatraemia can be an **artefact due to lipaemia** in systems which dilute the sample prior to assay (indirect). Measurement of **osmolality is an obsolete double-check**, because nowadays *all* samples are screened for lipaemia & sodium results vulnerable to artefact, are blocked.
- **Intoxication**. The non-specificity of serum osmolality is an advantage when abnormal, sometimes unknown, osmotically active solutes may be present eg. **ethanol** or **ethylene glycol** (antifreeze). The presence of these solutes are shown by an ↑ **osmolar gap** (the difference between measured & calculated osmolality). The cation concs. x2 estimates the contribution of the main counter anion (chloride). Not for the fastidious! But it works:

Measured osmolality – 2x[Na] – 2x[K] – [urea] – [glucose] = ≤ 10 mmol/kg (**osmolar gap**)

[ ] = serum concentration in mmol/L.

- To screen for **diabetes insipidus** & **SIADH**, see urine osmolality.
- The physiological **response to water deprivation** is monitored with urine osmo., body Wt. & serum osmo.

osmolality (urine) 3d 5mL fresh urine

Daily

50 – 1400 mmol/kg
Tietz

See comments, Na (serum), Na (urine conc) & osmo (serum).

- **See the helpful section on sodium in the Hospital Medical Handbook.**

Use

- Ix **hyponatraemia**.
- Screening for **DI** (diabetes insipidus).
- In the water **deprivation test**, to monitor the physiological response.

Background

- **Know the physiology of H_2O, Na & vascular vol. regulation** in health & disease. It enables common clinical situations to be appropriately managed.
- **Urine osmo. is more useful than serum osmo.** (especially if the urine Na conc. is measured too) for Ix Na & H_2O abnormalities eg. hyponatraemia & SIADH.
- To interpret urine osmo. results, **predict the physiological aims** based on history & **clinical examination** eg.

- Is ECF vol. ↓, ↑ or normal?
- What are the intakes & losses?
- What drugs are they on?
- Is the patient's medical condition associated with electrolyte & water problems?
- Do you predict that the kidneys are trying to retain or excrete Na & H_2O?

Joining the data together with knowledge of the physiology of water, volume & electrolyte regulation (see serum sodium) enables the cause(s) of ↑ & ↓ Na & the appropriate physiological response, to be predicted. Urine osmo. & sodium conc. results can then confirm your understanding or redirect it to other diagnoses.

- **HYPO natraemia** :

- **SIADH**, ECF vol. is normal or mildly ↑, urine osmo. is ↑ & urine Na conc. >30mmol/L despite the hyponatraemia (↑ ECF vol. is countered by release of natriuretic peptides).
- **Polydipsia**, the clinical & lab. findings are similar, except that **urine osmo. is ↓**.
- **Renal Na loss**, urine Na conc. is inappropriately ↑ & osmo. may be ↑ or ↓, but particularly, **ECF vol. is ↓**.
- **Extrarenal loss** or **↓ intake** sufficient to cause hyponatraemia, should be obvious. **ECF vol. will be ↓** (depending on fluid intake) & if the physiology is intact, there should be H_2O & Na conservation ie. **urine osmo. will be ↑** & **Na conc. < 30 mmol/L**
- **Oedematous states** eg. HF & ascites, the cause is complex & involves H_2O & Na retention, intake, drug effects & renal changes eg. due to age & heart failure. Urine testing adds little to clinical observations & predictions.
- **NB.** A mixture of common causes of hyponatraemia is more likely than a single uncommon one eg. SIADH, especially in **elderly** patients.

SUMMARY			
Cause of hyponatraemia	**Urine osmo.**	**Urine Na conc.**	**ECF vol.**
SIADH	↑	> 30 mmol/L	Not ↓
Polydipsia	↓	<, N or > 30	Not ↓
Renal loss	↑, N or ↓	> 30	↓
Extra-renal loss/↓ intake	↑	< 30	↓
Oedema/ascites	↑ or ↓	< or > 30	↑

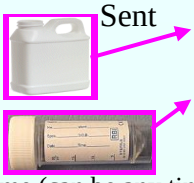
- **HYPER natraemia** :

- Excessive salt intake: rare
- Intake & metabolic production of water inadequate to replace normal or ↑ losses: much commoner eg. confused, elderly or weak patients, especially if they have ↑ loss eg. diarrhoea, fever.

Screening for diabetes insipidus. Avoid drinking after 10pm then measure the osmolality of the first urine passed next day (should be **> 450 - 500 mmol/kg**). If this is not reached or there is other evidence of DI, a formal water deprivation test may be needed.

oxalate 1d acidified urine 24h urine
See ethylene glycol, stone analysis, UA (urine) & vit. B6.

Children only



Normal	40 – 320 **u**mol/24h
1° hyperoxaluria	1.1 – 6.7 **m**mol/24h
Normal	0.005 – 0.080 mmol ox/mmol creat
St Helier Hospital	

- **Procedure**: Urinate in the toilet & note the time (can be any time). Collect all urine for the next 24h. The last one to collect is whatever can be passed at the noted-time next day.

Background

- Oxalate is the salt of oxalic acid & it **cannot be metabolised** by humans.
- **Excreted mostly in urine** & a little in faeces.
- **Sodium oxalate**, the soluble metabolic form, readily changes to the **less soluble calcium salt** in tissues & urine.
- Even in health, **urine is frequently supersaturated** with calcium oxalate ie. the concentration is higher than that at which crystals would form, but **ill-defined physico-chemical factors inhibit crystalisation** eg. citrate.
- **Ca oxalate is in 70% of all renal calculi** & crystals of it are found in the kidney in many renal diseases from glomerulonephritis to renal tubular acidosis.
- The **ref. range shows a 10 fold variation**, which makes measurement, especially as part of a **"stone-screen"**, insensitive & of **little value unless the patient is <40y old** (see below).
- Similarly, although the mean oxalate excretion in stone-formers is higher than in health, the wide scatter of results impairs the reliability of interpretation in individual patients.
- **Sources** of oxalate: **diet** 10-50%, metabolism of **ascorbic acid** 35-50%, metabolism of **glycine** 40%.

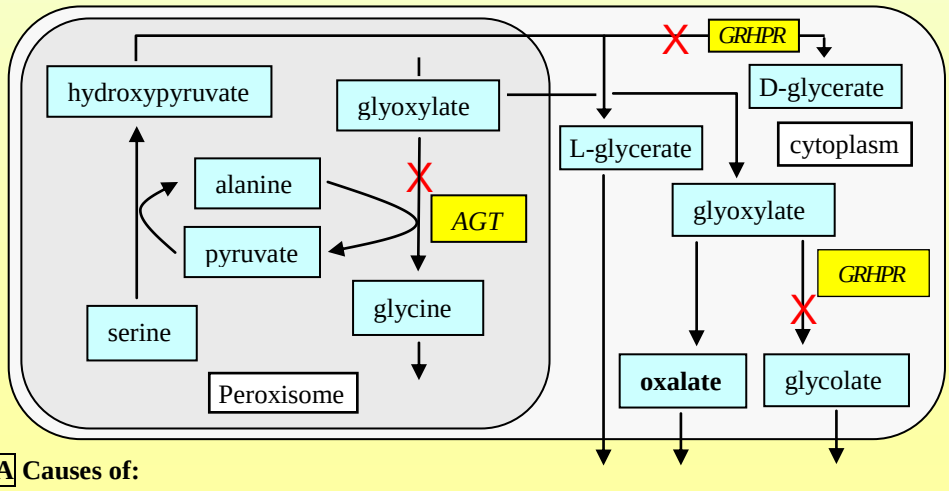
Causes of ↑ excretion

PRIMARY HYPEROXALURIA (OXALOSIS)

- **type I**. 1:100k (80% of cases) autosomal recessive defect of ***alanine glyoxylate aminotransferase*** (AGT) in liver peroxisomes → accumulation of glyoxylate & redirection into ↑ oxalate synthesis. **↑ urine oxalate** & **glycolate**.
- **type II**. 1:1m (10% of cases) an autosomal recessive defect of ***glyoxylate reductase/hydroxypyruvate reductase*** (GRHPR) in liver cytosol causes accumulation of glycerate & ↑ oxalate synthesis. **↑ urine oxalate** & **L-glycerate** but not glycolate.
- **type III**. (5% of cases) an autosomal recessive defect of ***4-hydroxy-2-oxoglutarate aldolase*** in liver mitochondria.
- In some cases these enzymes are normal. Probable defects of others.

Clinical effects • nephrolithiasis • renal failure • nephrocalcinosis • oxalosis (Ca oxalate deposits in bones, eyes, vasculature, heart etc), presenting from birth to mid-twenties. Presentation as late as age 40y has been reported.

See ethylene glycol DIAGRAM



ENTERIC HYPEROXALURIA
Causes of:

- chronic diarrhoea
- steatorrhoea
- exocrine pancreatic insuf.
- biliary cirrhosis
- biliary tract disease
- ileal resection
- jejuno-ileal bypass
- sprue
- coeliac disease

DIETARY HYPEROXALURIA **Causes of:**

- hi ascorbic acid intake • rhubarb, strawberries, spinach, tomatoes • oxalates in household products eg. cleaners
- hi intake of purines • hi intake of gelatine (boiled collagen, glycine rich) • ethylene glycol poisoning
- lo dietary Ca (less unabsorbable Ca oxalate formed) • pyridoxine (vit. B6) def. – a cofactor for AGT (see above)

IDIOPATHIC HYPEROXALURIA

- commonest type of hyperoxaluria in nephrolithiasis. ?due to heritable variation in oxalate absorption in gut.

Causes of ↓ excretion • renal failure

oxcarbazepine 3d 1.5mL

Sent

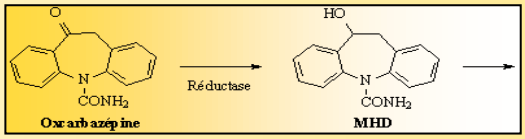
15 – 35 mg/L monohydroxy derivative (MHD)
St. Thomas' Hospital

See hydroxycarbamazepine (MHD) & therapeutic drug monitoring.

- **Pre-dose sample.**

Use

- Oxcarbazepine is rapidly metabolised to MHD, which accounts for most activity.
- **Routine monitoring** of oxcarbazepine, actually MHD, is **unnecessary** because of the predictable pharmacokinetics
- The oxcarbazepine dose is an adequate guide.
- **But**, MHD levels may be useful in:



- ↓ **GFR** (30% of MHD is excreted unchanged in urine)
- Ix of **compliance** or **loss/lack of clinical effect**
- Ix of **toxicity**.
- **Multiple drug therapy** eg. phenytoin & phenobarbitone ↑ clearance & ↓ MHD levels.

P

- P3NP
- pancreatic polypeptide
- paracetamol
- parathyroid hormone (PTH)
- PBG
- phenobarbitone
- phenytoin
- phosphate (serum)
- phosphate (24h urine)
- phosphate (urine conc.)
- placental ALP (PLAP)
- porphobilinogen (PBG)
- porphyrins (blood)
- porphyrins (faecal)
- porphyrins (urine)
- potassium (serum)
- potassium (urine conc.)
- potassium (24h urine)
- pregabalin
- primidone
- procollagen 3 N-terminal peptide (P3NP)
- progesterone
- prolactin
- protein (CSF)
- protein (serum total)
- protein (urine)
- protein/creatinine ratio (PCR)
- protein electrophoresis (serum)
- protein electrophoresis (urine)
- PSA (prostate specific antigen) free
- PSA (prostate specific antigen) total
- PSA ratio
- PTH

pancreatic polypeptide 0d 6mL + **aprotinin** Contact lab.
See gut hormone profile.

Sent

< 300 pmol/L
Charing Cross Hospital

paracetamol 2d 1.5mL
See ethanol, ethylene glycol, salicylate & TDM.

Daily

See the BNF
Roche Cobas ACETA method sheet V1

Sample timing is crucial.

- Knowing the **time between ingestion & blood sampling is crucial** to interpretetion of results eg. a harmless result at 4h post ingestion, could mean a devastating OD if it is actually 24h.
- If in doubt, **repeat** the assay. The 2nd result should be lower by the amount indicated by the decay profile between the 2 sample-times. A smaller fall than expected suggests a less recent OD.
- **Do not sample < 4h after ingestion** ie. before sufficient time for absorption from the gut.

Background

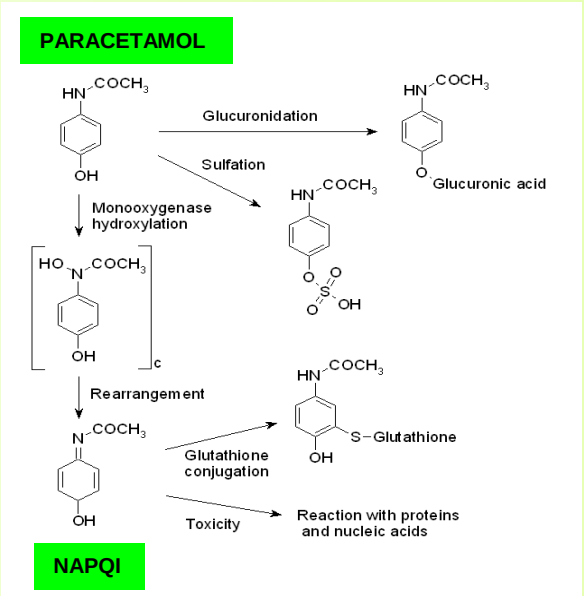
- Most ingested paracetamol is **inactivated** in the liver by **sulphate** & **glucuronide conjugation** before urinary excretion.
- A small amount is metabolised by hepatic cytochrome **P450** to the toxin N-acetyl-p-benzoquinone imine (**NAPQI**). **Reduced glutathione** is essential for inactivation, but supply is **limited**.
- **P450 is induced by**:

- chronic ethanol excess
- carbamazepine
- phenytoin
- phenobarbitone
- rifampicin
- St John's wort

- **Consequences** of induction:

- ↑ **NAPQI** production.
- **halving action-level** of paracetamol at which NAPQI production overwhelms inactivation.
- ↑ **risk of hepatotoxicity**.

- **Malnutrition** causes ↓ reduced glutathione & ↓ paracetamol level at which toxicity occurs.



parathyroid hormone (PTH) 3d 4mL

Twice a week.

1.6 – 6.9 pmol/L
Roche Cobas PTH method sheet V23

See ACE, Al, ALP, calcitonin, Ca, Ca adj, Ca (24h U), Ca (U conc), comments, Mg, PO_4, vit. D & Zn.

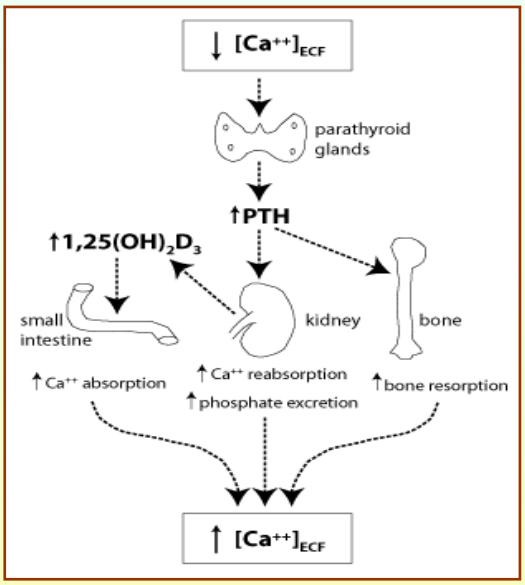
- **MUST have its own sample.**
- Measure Ca & PO_4 in serum taken at the same time.

Uses

- Ix of **hypercalcamia**
- In **CKD,** Ix of ↓ 1-hydroxylation of 25-OH vit. D which causes 2ndy hyperparathyroidism (PTH↑, Ca↓ or N)
- Plasma PTH is **insensitive for detecting vit D deficiency** (can be normal despite ↓ 25-OH vit. D).
- Ix **hypocalcaemia** (in hypoparathyroidism: Ca↓ & PO_4↑).

Background

- PTH (84aa) is secreted by the parathyroid glands in response to ↓ plasma ionised calcium conc.
- PTH restores Ca conc. by stimulating:
- **osteoclasts** to resorb bone, releasing Ca & PO_4.
- renal **tubular Ca uptake** & **PO_4 excretion**.
- renal hydroxylation of carbon 1 **of 25-OH vit. D** (activation) to form 1,25 dihydroxy vitamin D. This acts on gut mucosa to ↑ Ca + PO_4 uptake.
- PTH action ↑ plasma Ca to the set point & ↓ PO_4 (despite ↑ release from bone & ↑ uptake from the gut because PTH also ↑ renal PO_4 excretion).



Hyperparathyroidism - 3 types:

Serum Ca, PO_4 & U+E results at the time of the PTH result, are crucial to interpretation:

- **Primary: ↑ Ca** + ↓ PO_4 (or lower than GFR suggests) ie. autonomous secretion of PTH (level ↑ or N), not inhibited by hypercalcaemia eg. parathyroid hyperplasia & adenomas.
- **Secondary: ↓ Ca** + ↓ PO_4 (or lower than GFR suggests) ie. hypersecretion of PTH (level ↑) in response to ↓ plasma ionized Ca eg. due to deficiency of vit. D or its activation by 1-hydroxylation in CRF.
- **Tertiary: ↑ Ca** + ↓ PO_4 (or lower than GFR suggests) ie. autonomous secretion of PTH (level ↑ or N) developing after chronic 2ndy hyperparathyroidism. It presents particularly after renal transplantation.

Hypoparathyroidism

- **Less common** than hyperfunction & most often after **parathyroidectomy** for primary hyperparathyroidism.
- Typically, serum shows **↓ Ca** + **↑ PO_4** due to: - ↓ Ca release from bone &
  - ↓ Ca & ↑ PO_4 uptake by the renal tubule.

Hypercalcaemia

- Malignant bone invasion eg. myeloma. PTH ↓
- .. humoural hypercalcaemia ie. PTHRP secretion. PTH ↓
- Primary & tertiary hyperparathyroidism. PTH ↑.
- Familial hypocalciuric hypercalcaemia. PTH in the upper half of the ref. range or a little ↑ .

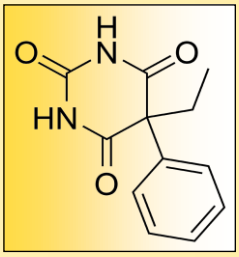
phenobarbitone 3d 1.5mL

Sent

See carbamazepine, drugs & TFTs, lamotrigine, phenytoin, primidone, sirolimus, TDM & zonisamide.

- **Pre-dose sample.**

Adult	≤ 40 mg/L
Neonate	≤ 30
St Helier Hospital	



Use

- Routine **monitoring is less recommended** these days, but levels are still useful for:

- Ix toxicity
- Ix compliance
- Multidrug therapy if the other drugs change phenobarbitone clearance
- Ix difficulty with establishment of therapeutic effect or loss of it.

Background

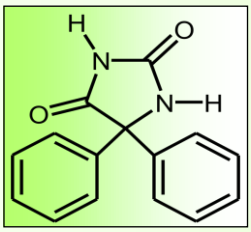
- Synthesised over 100 years ago, but its only remaining substantial use is treatment of **epilepsy**.
- Hepatic metabolism, mainly hydroxylation by CYP2C19, then **glucuronidation & renal & faecal excretion**.
- CYP2C19 & other cytochrome P450 enzymes are **induced** by phenobarbitone, which **hastens elimination** of itself & **co-prescribed drugs** eg. carbamazepine, phenytoin, lamotrigine, valproate & zonisamide.
- Phenobarbitone is used to induce **hepatic *glucuronyl transferase***, which is deficient in type 2 Crigler-Najjar syndrome & causes an **unconjugated hyperbilirubinaemia**.

phenytoin 3d 1.5mL

Daily

See carbamazepine, drugs & TFTs, lamotrigine, phenobarbitone, sirolimus, TDM, valproate & zonisamide.

- **Pre-dose sample.**



Therapeutic range	10 - 20 mg/L
Toxicity unusual	<15
Nystagmus	>20
Ataxia	25 - 30
Somnolence/dysarthria	>40
Roche Cobas PHNY2 method sheet V9	

Use

- **Optimising Px**. Serum levels correlate with clinical response & freedom from side effects, better than the dose.
- The need for monitoring most other anticonvulsants is not so clear.

Background

- Phenytoin, like carbamazepine, inhibits voltage-gated Na channels, which leads to ↓ neuronal excitability & ↓ spread of epileptic activity.
- Assays are necessary because its **absorption** is **slow, variable** & **vulnerable to tablet formulation**.
- Also, **clearance** by hepatic catabolism **saturates** at therapeutic levels ie. above this point, small ↑ dose or ↓ metabolic capacity eg. due to **intercurrent illness**, can give disproportionately big ↑ in serum phenytoin levels.
- The **saturating dose cannot be predicted**. It varies with inheritance & can be modified by other drugs eg. carbamazepine.
- When titrating the dose, it may be necessary to test as often as 12hrly eg. iv therapy for status epilepticus. More usually, **equilibration** occurs 1 – 2 w after starting or changing an oral dose.

phosphate (serum) 3d See profiles

See ACE, Al, ALP, Ca, Ca adj, comments, creatinine, eGFR, **MRI**, PO_4 (24h urine), PTH, stone analysis & vit. D.

Daily

Age	Female (mmol/L)	Male (mmol/L)
Prem.	1.30 - 2.80	1.30 - 2.80
1 - 30d	1.40 - 2.50	1.25 - 2.25
1 - 12m	1.20 - 2.10	1.15 - 2.15
1 - 3y	1.10 - 1.95	1.00 - 1.95
4 - 6y	1.05 - 1.80	1.05 - 1.80
7 - 9y	1.00 - 1.80	0.97 - 1.75
10 - 12y	1.05 - 1.70	1.05 - 1.85
13 - 15y	0.90 - 1.55	0.95 - 1.65
16 - 18y	0.80 - 1.55	0.85 - 1.60
Adult	0.81 - 1.45	0.87 - 1.45
Roche Cobas PHOS2 method sheet V5 & Roche Ref. Ranges for Adults & Children.		

Use

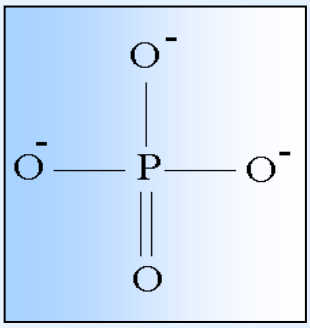
- Ix Ca abnormality, CKD, AKI, therapy of hyperglycaemia

Background

- **88% of total body PO_4 is in bone** as calcium phosphate.
- The rest is in phospholipids, nucleic acids, metabolic intermediates, ATP, ADP, AMP etc.

Causes of ↓

- time of day (lowest in pm., highest late am.)
- insulin released by carbohydrate meals & dextrose ivi
- exogenous insulin to treat DKA
- ethanol excess
- hyperparathyroidism (with ↑ Ca in primary, ↓ in 2ndy)
- vit. D deficiency (↓ Ca too)
- renal tubular defects of PO_4 reabsorption
- total parenteral nutrition



Causes of ↑

- ↓ GFR
- gross haemolysis of sample ex-vivo
- DKA
- hypoparathyroidism (serum Ca ↓)
- acromegaly
- hypervitaminosis D (serum Ca ↑)
- children normally have higher levels than adults

phosphate (24h urine) 3d 24h urine

See Ca (24h urine), Ca (serum), PO_4 (serum), stone analysis & uric acid (urine).

Daily

13 – 42 mmol/24h
Roche Cobas PHOS2 method sheet V5

Use

- **Little value** though commonly requested to Ix nephrolithiasis.
- Serum Ca, PO_4 & PTH are **more useful**.
- **Wide variation** with intake

- **Procedure**: Urinate in the toilet & note the time (can be any time). Collect all urine for the next 24h. The last one to collect is whatever can be passed at the noted-time next day.

phosphate (urine conc.) 3d acidified 20mL

See PO_4 (24h urine)

Daily.

Use

- **Little value.**
- **Highly dependent on intake & dilution.**
- Serum Ca, PO_4 & PTH are **more useful**.

Morning urine	13 – 44 mmol/L
Roche Cobas PHOS2 method sheet V5	

porphobilinogen (PBG) 0d 5 mL

See porphyrins (blood) & urobilinogen.

- **Protect from light** with aluminium foil.

Sent

Ref. & comment sent with result
UHW, Cardiff

porphyrins (blood) 0d 2 full tubes (small children 1mL)
See abbreviations, bilirubin, hemopexin, PBG, porph faecal, porph urine & urobilinogen.
- **Protect from light with aluminium foil.**

DIAGRAM of haem synthetic pathway**.**

Sent

Refs & comment sent with result
UHW, Cardiff

Background

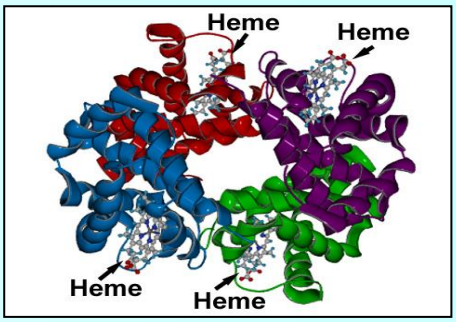
- Porphyria is a **congenital** disorder of haem synthesis in which toxic & light sensitive precursors accumulate.
- **Acquired** types exist too eg. PCT & ↓ *PBG synthase* (*ALA dehydratase*) activity can be caused by lead poisoning.
- Categorised as **hepatic** or **erythropoietic** porphyria depending on the *most* affected organ ie. liver or bone marrow.
- In reality there is **overlap** eg. EPP causes hepatic dysfunction too.
- Clinical phenomena relate to which enzyme, residual activity, drugs & predisposing disease (below & DIAGRAM).
- **For diagnosis, send all 3** – blood, urine & faeces. **To exclude acute porphyria, urine will do**.

Clinical presentation	Specimens	Comments
ACUTE neurological attacks (suspected **AIP, VP, HCP** & **ADP**) ie. abdo. pain, vomiting, neuropathy, psychiatric symptoms etc.	**urine** **faeces** **blood**	Urine alone is adequate to exclude a current attack or to monitor patients *known* to have porphyria.
ACUTE photosensitivity (suspected **EPP**) ie. erythema, pain, pruritus, oedema on exposure to sunlight, without bullae & little or no scarring.	**blood**	Urine & faeces are of no value
CHRONIC skin lesions (suspected **PCT, VP, HCP** & **CEP**) ie. bullae, scarring, skin fragility, milia, pigmentation, hypertrichosis etc.	**urine** **faeces** **blood**	Blood on its own is adequate if the disease is active at the time. To monitor PCT patients, urine sufficient.
Family studies	Seek advice from the physician caring for the index case after their diagnosis has been established.	

ABBREVIATIONS

AIP	= acute intermittent porphyria
VP	= variegate porphyria
HCP	= hereditary coproporphyria
ADP	= ALA-dehydratase porphyria (natural + Pb toxicity)
EPP	= erythropoietic protoporphyria (protoporphyria)
PCT	= porphyria cutanea tarda
CEP	= congenital erythropoietic porphyria
urine	= 20 mL fresh urine in a sterile universal container
faeces	= 5 – 10g faeces
blood	= 10 mL EDTA blood (1mL min. in children)

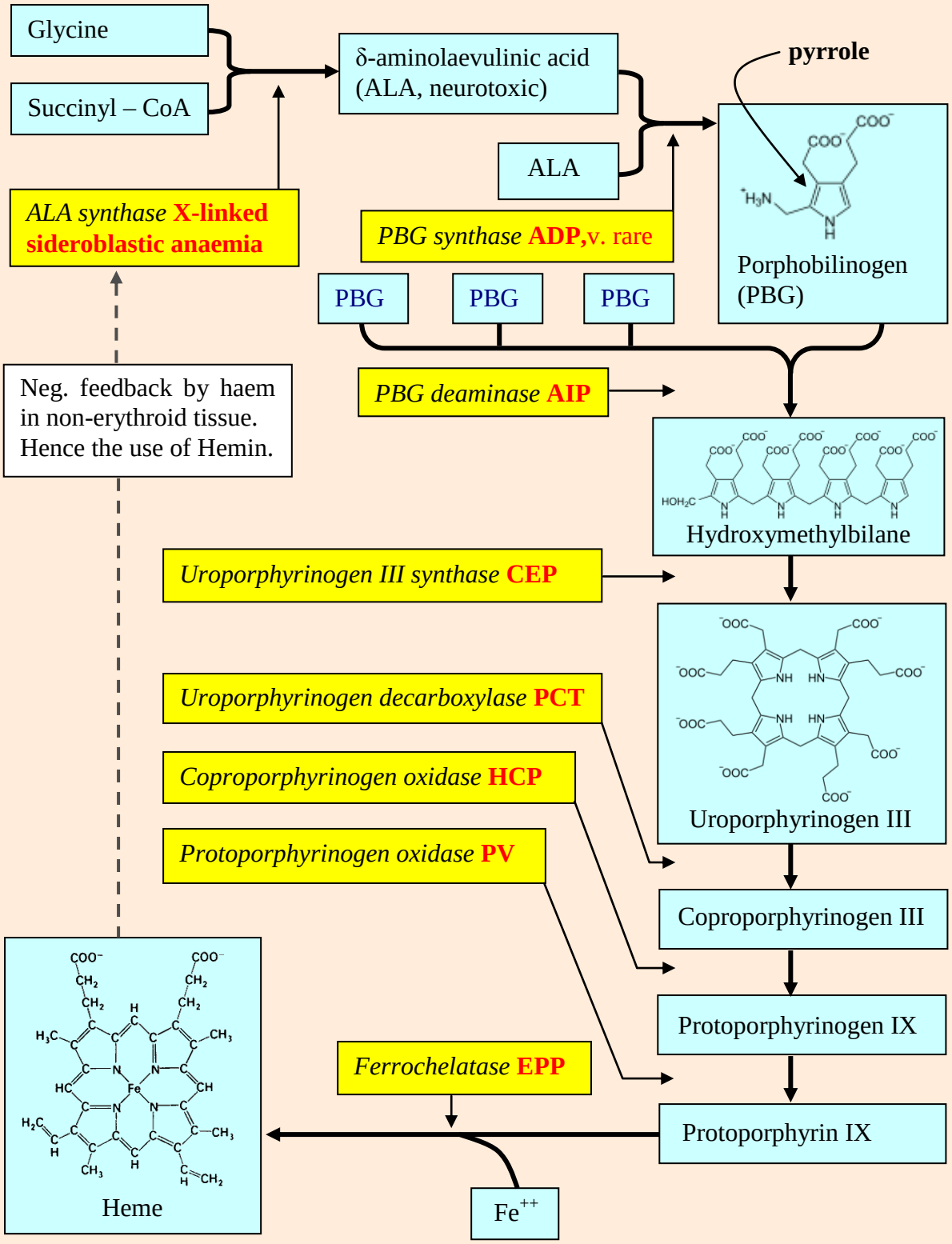
Haemoglobin



BIOCHEMICAL FINDINGS in porphyria

	HEPATIC							ERYTHROPOIETIC	
	AIP		PV		HCP		PCT	CEP	EPP Liver impact too
	Acute	Latent	Acute	Latent	Acute	Latent			
CLINICAL EFFECTS									
Abdo. / neuro.	+	–	+	–	+	–	–	–	–
Skin	–	–	+	+	Rarely	rarely	+	+	+
BIOCHEMISTRY									
Urine PBG	+	+	+	–	+	–	–	–	–
Urine porph.	+	–	+	–	+	–	+	+	–
Faecal porph.	–	–	+	+	+	+	–	+	+

Haem synthetic pathway & location of enzyme defects. Abbreviations, porphyrins & vit. B6



porphyrins (faecal) 0d 10g

See porphyrins (blood) & porphyrins (urine).

- **Protect sample from light with aluminium foil**.

Sent

Ref & comment sent with result

UHW Cardiff

porphyrins (urine) 0d 20mL

See porphyrins (blood) & porphyrins (faecal).

- **Protect sample from light with aluminium foil**.

Sent

Ref & comment sent with result

UHW Cardiff

potassium (serum) 3d See profiles
See aldosterone, anion gap, comments, cortisol, creatinine, digoxin, eGFR, haemolysis, MRI, Na, renin & urea.

Daily

3.5 – 5.1 mmol/L
Roche Cobas ISE indirect Na-K-Cl Gen.2 method sheet V7

Children	premature	3.2 - 4.6 mmol/L
	1d - 4w	3.6 - 6.1
	1m -12m	3.6 - 5.8
	>1y	3.1 - 5.1
Adults	≤60y	3.3 - 5.1
	>60y	3.7 - 5.4
Roche, reference Ranges for Adults & Children 2004		

Categories of causes of abnormal K:

- **Artefact**
- **Input**
- **Output**
- **Distribution**:

Causes of ↑

artefact
- K EDTA contamination – **NEVER** take FBC sample before chemistry.
- "fist-clenching"
- degraded sample – old, shaken, ejected through needle, too hot, too cold
- haemolysis – **NEVER** eject blood through a needle. **AVOID** cannulae (be gentle if you cannot).
- thrombocytosis - platelets release K on clotting (normal ↑ 0.2-0.4 mmol/L). Check by comparing Li hep. vs. clotted blood K results, if necessary.
- leukocytosis

↑ input
- K supplements, ivi, & LoSalt especially if GFR is ↓.

↓ output
- ↓ GFR
- hypoaldosteronism
- hyporeninaemic hypoaldosteronism
- NSAIDs, ACEIs, ARBs, K-sparing diuretics.

redistribution
- acidaemia – intracellular K exchanges with ECF H^+
- beta blockers
- severe tissue injury

Causes of ↓

artefact
- none, except "drip arm" contamination with low K ivi

↓ input
- chronic starvation. Aggravated by 2ndy hyperaldosteronism if ECF vol ↓.

↑ output
- **gut**: diarrhoea, vomiting, intestinal fistulae, laxative abuse
- **kidney**: diuretics, hyperaldosteronism, Cushing's, ectopic ACTH, licorice, RTA, renal tubular disease, Fanconi syn., Bartter's syn. (normotensive, rare), Liddle syn. (hypertensive, v. rare).

redistribution
- insulin (exogenous, or endogenous in response to dextrose ivi.)
- alkalaemia
- beta stimulants
- hyperadrenalism

potassium (urine conc.) 3d 20mL

Daily

See serum potassium.

25 – 125 mmol/L
Tietz

Use

- **Not a useful test** because the potassium concentration in urine is highly dependent on intake & dilution

potassium (24h urine) 3d 24h urine

Daily

See serum potassium.

Use

The variability of potassium excretion & its dependence on intake mean that the **results have little value**.

	Female	Male
6 - 10y	8 - 37	17 - 54 mmol/24h
10 - 14y	18 - 58	22 - 57
adult	25 - 125	25 - 125

Tietz

pregabalin 3d 3mL **No gel**

Sent

See gabapentin & therapeutic drug monitoring.

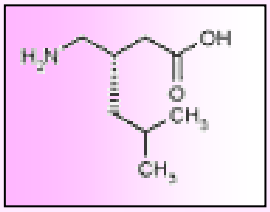
- **Pre-dose sample.**

See report
St. Thomas's Hospital

Use

- **Routine monitoring is unnecessary** because of linear pharmacokinetics.
- But levels may help in Ix of compliance, toxicity & loss of clinical effect or failure to establish it .

Background



- Used to treat **epilepsy** & **neuropathic pain**
- A structural analogue of **GABA** - like gabapentin.
- Binds **voltage gated Ca channels**, inhibiting excitatory neurotransmitter release
- Well absorbed
- **Half-life** = 6h
- 90% excreted unchanged in **urine.**

Causes of ↑

- ↓ GFR

Biochemical side effects

- ↑ CK

primidone 3d 1.5mL

Sent

See phenobarbitone & TDM.

- **Pre-dose sample.**

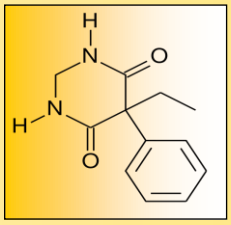
≤ 11.0 mgL
St Helier Hospital

Use

- **Measurement of phenobarbitone is more useful** than assay of primidone itself.

Background

- A **prodrug** metabolised in the **liver** to the **active molecule, phenobarbitone**.



procollagen 3 NP (P3NP) 2d 4mL **No gel**  Sent
(procollagen-3 N-terminal peptide)

Use

- Assessment of on-going **hepatic fibrosis** due to long term **methotrexate** (MTX) therapy.

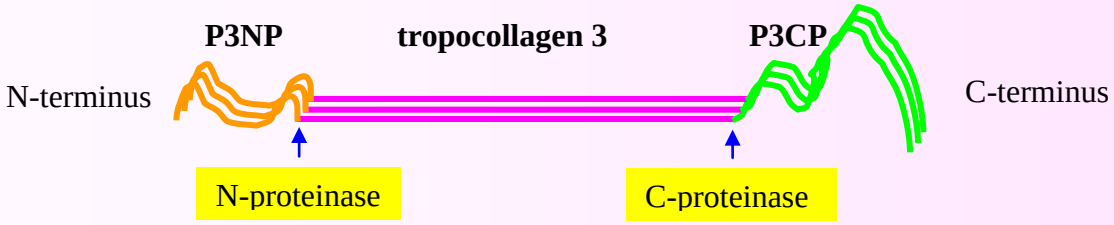
Test

- Measure after 1 – 1.5g of MTX in total after the start:
- **if normal**, repeat assay after another 1g or annually
- **if** ↑, ↓ dose & measure P3NP 3 months later.

	Female (ug/L)	**Male** (ug/L)
0 - 2 y	10 - 50	10 - 50
2 - 4 y	5 - 15	5 - 15
5 - 10 y	5 - 10	5 - 10
11 - 14 y	8 - 15	5 - 10
15 - 19 y	2 - 8	8 - 20
20 - 65 y	1.7 - 4.2	1.7 - 4.2
Sheffield Protein Reference Unit		

Background

- P3NP is the **N-terminal fragment of procollagen-3** (the precursor to collagen type 3) which is released in equimolar amounts during extracellular maturation after secretion ie. P3NP is a **marker of NEW fibrosis**.



- **Old fibrosis will not ↑ P3NP**, even if extensive.
- Collagen 3 is relatively abundant in hepatic fibrosis
- P3NP assay is a substitute for liver biopsy for detection of fibrosis due to large accumulated doses of MTX.
- ↑ by other causes of
- Serum P3NP peaks during normal **growth** in childhood & puberty.

Causes of ↑

- Hepatic fibrosis which is on-going eg. MTX, alcoholic liver disease, sclerosing cholangitis.
- Non-hepatic fibrosis eg. myelofibrosis, systemic sclerosis.

progesterone 2d 1.5mL
See FSH, hCG, LH, **MRI**, oestradiol & prolactin.

Daily

Women	Follicular phase	0.6 - 4.7 nmol/L
	Ovulation	2.4 - 9.4
	Luteal phase	5.3 - 86.0
	day 21	>30.0
	postmenopausal	0.3 - 2.5
Men		0.7 - 4.3
Roche Cobas Progesterone II method sheet V16 (5^{th} - 95^{th} centiles)		

- **State day of menstrual cycle in the request.**

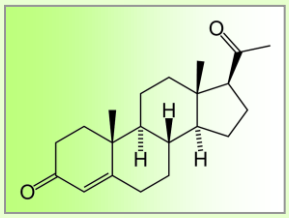
Use

- Day 21 progesterone is a **marker of ovulation**.
  Actually, corpus luteum formation, which can occur without ovulation having taken place.
- **Monitoring the conceptus** eg. in the management of **ectopic pregnancy** & **miscarriage.**

Background

- Progesterone is **low in the follicular phase** of the menstrual cycle & starts to ↑ **just before ovulation**. See figure.
- ↑ further in the luteal phase as the **corpus luteum**, which forms at the site of the shed ovum, develops & synthesises more progesterone.
- **At this early stage** of what might become pregnancy, **the main function of progesterone is to stimulate development of the secretory endometrium** so that it is ready for intrauterine implantation of a fertilised ovum when it arrives (a blastocyst by then).
- By **35 days**, the **placenta takes over** secretion of progesterone which continues to be needed**:**

- for endometrial function
- to inhibit premature uterine contractions
- for milk gland development in concert with oestradiol.



- **In pregnancy**, progesterone levels increase progressively to term.
- **In the absence of implantation** & a placenta to synthesise hCG to maintain the corpus luteum via LH receptors, progesterone **levels** ↓ as the corpus luteum declines, with the result that the secretory endometrium degenerates & **menstruation ensues** (see figure).

prolactin 1d 1.5mL Daily

See FSH, hCG, LH, macroprolactin, **MRI**, oestradiol, progest. & TRH test

Female (non-pregnant)	102 - 496 mU/L
Male	86 - 324
Roche Cobas Prolactin II method sheet V6 (2.5th – 97.5th cent)	

- **"Measurement of serum prolactin is not recommended for the diagnosis of epilepsy"** NICE CG20. Poor specificity & sensitivity (too many false + ves & false – ves , respectively).

Background

- a **198 a.a. glycoprotein** secreted by the **lactotropes** of the anterior pituitary.
- Secretion is inhibited by **dopamine** from hypothalamic neurones.
- Circulates as biologically active **monomers** (80%) & inactive **dimers** (5 - 20%) & **tetramers** (0.5 - 5%)
- Aggregates of prolactin & IgG (**macroprolactin**) may also be present. They are harmless but cause result artefacts which falsely suggest hyperprolactinaemia & can lead to diagnostic confusion & needless investigation.
- Prolactin stimulates growth & activity of milk ducts & glands rather than breast tissue ie. hyperprolactinaemia causes **galactorrhoea. Oestrogens** cause breast enlargement, both physiological & pathological (gynaecomastia).
- The ↑↑ oestradiol & progesterone levels of **pregnancy** stimulate lactotrope growth & secretion of prolactin, which prepares the breast for **lactation**.
- Hyperprolactinaemia **inhibits** ovarian steroidogenesis & secretion of **FSH** & **LH**, particularly their cyclic regulation, which can result in **infertility** (anovular cycles & reduced sperm count), **amenorrhoea**, oligomenorrhoea & **hypogonadism**, in addition to the familiar galactorrhoea.

Causes of ↑

- stress
- oestrogens
- pregnancy
- suckling
- loss of dopaminergic tonic inhibition eg. antipsychotic drugs
- prolactinoma
- primary hypothyroidism (untreated).
- TRH test.
- macroprolactinaemia

Causes of ↓

- dopamine agonists eg. levodopa & ergot derivatives
- hypopituitarism.

protein (CSF) 3d 0.5mL Daily

See CSF glucose, CSF oligoclonal bands & xanthochromia.

0.15 – 0.45 g/L
Roche Cobas TPUC3 method sheet V5

Use Ix disease of the meninges & brain.

Causes of ↑

- ↑ **permeability** of the blood-brain-barrier eg. due to inflammatioin: meningitis, encephalitis, polyneurtis, MS.
- **Tumours** eg. meningioma, neuroma
- **Froin's syndrome**: extreme ↑ in lumbar CSF protein content below the site of spinal cord compression by tumour
- **Traumatic LP**.

protein, total (serum) 3d 1.5mL

See albumin, **MRI** & protein electrophoresis.

Daily

Children	**premature**	36 - 60 g/L
	neonate	46 - 70
	1w	44 - 76
	7m - 1y	51 - 73
	1y - 2y	56 - 75
	>3y	60 - 80
Adults		64 - 83
Roche Cobas TP2 method sheet V5		

Use • Adds little to specific assays eg. LFT, Igs & albumin.

Background

- **The sum of serum proteins** eg. albumin, Igs, complement, transferrin, ferritin, inflammatory activators & inhibitors etc. minus clotting factors because the sample is serum.
- They come mainly from the liver, plasma cells, spleen, bone marrow & lymph nodes.
- The difference between the albumin & total protein concentrations is approx. the concentration of **globulins**.
- Though total protein assay is **cheap**, in practice it supplies **little information that is not apparent clinically**.

Causes of ↑

- prolonged tourniquet use during venesection
- myeloma
- dehydration ++
- upright posture

Causes of ↓

- blood loss
- surgical recovery
- burns
- nephrotic syndrome
- bedrest
- malnutrition

protein (24h urine) 3d 24h urine

See ACR, protein:creatinine ratio & protein electrophoresis (urine).

Daily

≤ 140 mg/24h
Roche Cobas TPUC3 method sheet V5

Use

- Largely **replaced by ACR & PCR** because of the difficulty in collecting an accurately timed sample.
- **Procedure**: Urinate in the toilet & note the time (can be any time). Collect all urine for the next 24h. The last one to collect is whatever can be passed at the noted-time next day.

protein:creatinine ratio (PCR) 3d EMU

Daily

0 – 45 mg/mmol
NICE CG73

See albumin/creatinine ratio & protein (24h urine).

Use

- A more sensitive test for proteinuria than dip-sticks, without the inconvenience & unreliability of 24h collections.

Background

- Proteinuria has been known to be a **marker of nephropathy**, for a long time.
- Assay of protein mass in a **24h urine** collection was the quantitative standard for clinical use until recently.
- Unfortunately, in real life, timed collections are **inconvenient & hard to collect precisely**.
- The variability of PCR measurements is offset by the **ease of repetition**.
- PCR is for use in **established proteinuria** eg. ACR confirmed as ≥ 30 mg albumin/mmol creatinine.

The albumin/creatinine ratio (**ACR**) was formerly recommended only for monitoring diabetic nephropathy. But in 2008, NICE in CG73 recommended ACR as the 1st line test for proteinuria **in non-diabetic patients** at risk of CKD, because it is **more sensitive than PCR**.

A *ROUGH* GUIDE TO EQUIVALENT RESULTS			
dip-stick	**PCR** mg/mmol	**ACR** mg/mmol	**24h protein** g/24h
– ve	< 15	0 - 30	< 0.15
trace	15 - 44	2.5 - 30	0.15 - 0.45
+ or ++	45 - 449	30 - 300	0.45 - 4.50

protein electrophoresis (serum) 3d 1.5mL Daily

See A1AT, albumin, BJP, BJP quant., CDT, cryoglobulins, free light chains, Igs, **MRI**, CSF oligos., tau prot. & tot. prot.

comment/quantitation

Use

- Detection & quantitation of **myeloma paraprotein**. Send **spot urine too** to test for **BJP**, if screening for myeloma.
- Past uses are now better served by specific tests eg. CRP, LFTs, Ig assays, α1AT & imaging.

Background

- On a thin sheet of **agarose gel** (modified seaweed polysaccharide) in **buffer** to control the pH & protein ionisation, serum proteins have a net **– ve charge** (anionic) & move to the + ve electrode (anode) in an electric field.
- The **speed of migration of a protein** depends on:

- charge
- molecular size & conformation
- physico-chemical interactions with the solid & liquid phases eg. viscosity, sieving & hydrophobicity.

- **Serum proteins separate into 6 groups or bands**, which are made visible by non-specific staining**:**

- **albumin** - the fastest moving band & closest to the anode.
- **α1** band - mostly α1 antitrypsin
- **α2** band - haptoglobin, α2 macroglobulin
- **β1** band - transferrin, LDL
- **β2** band - C3 complement
- γ region - slowly migrating immunoglobulins, especially types IgM & IgG. IgG & IgA have fast migrating forms which speed ahead as far as the β1 & α2 bands, respectively.

- The electrophoresis strips are **examined by eye**, optically **scanned to quantitate** abnormal proteins (**paraproteins**) & if necessary, re-run & "stained" with antisera (**immunofixation**) to identify the type of immunoglobulin present in the paraprotein & to determine if it is of only one type ie. if it is **monoclonal**.

Causes of changes to bands:

albumin	↓ • any cause of hypoalbuminaemia. Harmless congenital variants may migrate at a different speed. Heterozygotes have 2 bands – bisalbuminaemia. ↑ • prolonged phlebotomy tourniquet usage • dehydration.
α1	↓ • (isolated) in α1AT deficiency • nephrosis (↓albumin, ↓α2 & ↓γ too). ↑ • with ↑α2 in inflammation & tissue injury of any cause. ↑ γ region too in chronic state.
α2	↓ • nephrosis (↓albumin, ↓α1 & ↓γ too) ↑ • with ↑α1 in inflammation & tissue injury of any cause. ↑ γ region too in chronic state.
β1	No clear characteristic changes. A paraprotein at this site eg. IgA, can look like a normal band.
β2	.. ..
γ	↓ - **diffuse** Ig loss eg. myeloma • malnutrition • congenital deficiency (IgA commonest). ↑ **diffuse** Ig ↑ eg. inflammation • cirrhosis. Many different antibodies are present but they cannot be resolved as individual bands. If ↑↑, β & γ bands may merge (β-γ fusion). **monoclonal** paraproteinaemia: overproduction of a single antibody type by a B-cell clone eg. myeloma, MGUS, B – cell lymphoma. See BJP. **oligoclonal response**. With age & immunodepression, sometimes a more limited antibody response to inflammation produces a few distinct γ bands rather than the usual diffuse ↑. **NB.** This is **different from CSF oligoclonal bands.**

protein electrophoresis (urine) 3d 20mL EMU
See ACR, BJP, free light chains & protein (24h U).

Daily

Interpretation

PSA (prostate specific antigen) free 3d 1.5mL
See PSA total

Tuesdays & Fridays

Use

- For use by **urologists** in combination with other resources for **Ix carcinoma of the prostate**.

Background

- Approximately **30%** of circulating PSA (34 kDa prostate-specific **serine protease**) is **not complexed with serpins** (serine protease inhibitors) eg. α1-antichymotrypsin & α2-macroglobulin. This is termed "**free-PSA**".
- Biopsy + ve **carcinoma of the prostate** is associated with a ↓ **free-fraction** of total PSA.
- This is used to shed light on the meaning of total–PSA results in the "**grey zone**" between the upper reference limit & levels associated with significant frequency of prostatic carcinoma eg. 4 – 20 ug/L.
- Unfortunately, free PSA gives **only a *relative*** ↑ in sensitivity & specificity for detection of carcinoma compared with total-PSA, because **levels in health & cancer overlap considerably**. See what's a ref. range.
- This means that **a simple reference range cannot be defined**, in terms of cancer risk, without qualification by additional factors eg. clinical findings & ultrasound scans.
- Thus free-PSA assay has to be **limited to the urology team** who have the necessary algorithms & data from additional prostatic investigations with which to put free-PSA results in context & to detect cancer without an excessive frequency of **false + ves**.

PSA (prostate specific antigen) total 3d 1.5mL
See eGFR, K, **MRI**, Na, PSA free & urea.

- **Avoid when false +ve risk high** (see Causes of ↑ below).

Daily

Age (y)	**PSA**
<40 y	1.4 ug/L *(95th centile)*
40 - 50	2.0
50 - 60	3.1
60 - 70	4.1
≥ 70	4.4
Roche Cobas total PSA method sheet V4	

Use

- **Monitoring** histologically diagnosed prostatic carcinoma.
- Used cautiously, for **screening** men at high risk of ca. prostate

Background

- PSA is a 34kDa glycoprotein **serine endoprotease** ie. catalysis involves a key serine residue in its active site & peptide bonds are cleaved within proteins rather than at their termini.
- Secreted by the epithelium of the **prostatic ducts** & **acini.**
- In plasma, PSA irreversibly forms **complexes with protease inhibitors** such as α1-antichymotrypsin.
- About 30% of circulating PSA is free (not complexed), but it too is proteolytically inactive. Therefore, despite being an enzyme, **PSA immunoreactivity** is measured rather than catalytic activity.
- PSA is tissue-specific but **not pathology-specific**.
- There are **false + ves** & **false – ves** for carcinoma ie. **PSA cannot be used to screen a low risk population**.
- But it is a useful **marker of recurrence & response to treatment** of histologically proven prostatic carcinoma.
- **NB**. PSA-secretion can be **"lost"** by tumours if they become less differentated.
- **Plasma half-life = 3d** ie. ↑ levels should fall to the ref. range within 2 – 3 weeks of radical prostatectomy.
- **Women** form PSA too in paraurethral, perianal & apocrine sweat glands & breast tissue.

Causes of ↑ total PSA

( ) = wait before testing because of risk of false +ve

- prostatic cancer (MRI = 2 w)
- manipulation (1 w)
- trauma (6w)
- ejaculation (48h)
- benign prostatic hypertrophy
- cystoscopy (1w)
- colonoscopy (1w)
- exercise ++ (48h)
- prostatitis (may take months to ↓)
- urinary retention (1w)
- UTI (may take months to ↓)
- prostate Bx (6w)

Q

quinine

quinine 3d 3mL

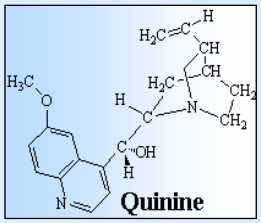
Sent

10 – 15 mg/L
St. Thomas' Hospital

See therapeutic drug monitoring.

Use

- Optimisation of treatment of falciparum malaria with quinine.



Background

- Powdered cinchona tree bark was used for millenia by South American people to **treat malaria** before spanish colonists brought it to Europe in 1630.
- In 1820 quinine, the active ingredient of "jesuit powder", was purified.
- Drug resistant **falciparum malaria** is still treated with quinine although new drugs eg. **arte-ether** developed from the chinese herb artemisinin, provide safer & more effective alternatives.

R

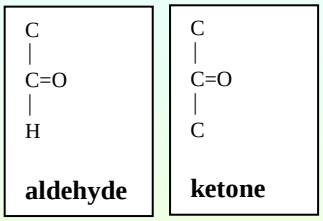
reducing substances (faeces) 1d a fresh piece Daily

See elastase, oligosaccharides, reducing substances (urine) & sugar chromatography.

Refs & comment sent with result

+ ve screen samples sent to GOSH

- The test needs its **own sample.**
- Specimen **must be fresh** (bacteria degrade sugars).
- Exclusion diets can give **false negatives.**



Use

- Ix of chronic **diarrhoea** in children for defects of digestion/absorption.

Background

- Some sugars called **aldoses** eg. **glucose,** in their linear form contain an **aldehyde** group (a carbonyl group, C=O, bonded to 1 carbon & 1 hydrogen atom ie. a formyl group) at the end of a carbon chain.
- Others (**ketoses**) eg. **fructose** have a **ketone** group (the carbonyl group (C=O) is bonded to 2 carbons).
- In **alkaline copper** sulphate solution (**Benedict's test**), these groups can **reduce** (donate electrons to) Cu^{++} ions to form Cu^{+} & copper (I) oxide, a red/brown precipitate.
- Other sugars reduce too eg. **lactose** & **maltose**, but **not sucrose** (disaccharides).
- A **weak + ve result needs to be confirmed** in a 2nd sample before being sent for chromatography.
- **chromatography** at a specialist lab., is used to identify the substances present, as with urine reducing substances.
- Defects of **absorption** or **mucosal saccharidases** eg. **lactase**, cause sugars to reach the colon where bacteria metabolise them to acids & gas. Undegraded sugar gives faeces reducing ability.
- Stools containing malabsorbed carbohydrate are characteristically **watery & frothy**.

Interpretation

- Diarrhoea of **any cause** can give + ve results due to incomplete absorption.
- **Lactase deficiency** gives a + ve result. Chromatography reveals **lactose, glucose** & **galactose**.
- **Glucose-galactose malabsorption** causes a + ve too, but **only glucose & galactose** are found (**not *lactose***).
- **Hydrogen breath test** after lactose ingestion, is better for Ix lactase deficiency in older children & adults.

reducing substances (urine) 1d 2mL fresh urine Daily

See Gal-1-PUT, oligosaccharides, reducing substances (faeces) & sugar chromatography.

Refs & comment sent with result

+ ve screen samples sent to GOSH

- Specimen **must be fresh** (bacteria degrade sugars).
- Exclusion diets can give **false –ves.**

Use

- An insensitive screen for a variety of conditions (see below).

Background

- For details of what a "reducing sugar" is, please see faecal reducing substances.
- **Galactosaemia** - An **insensitive** screen. Galactose may be absent if intake has been minimised. Direct assay of galactose-1-phosphate uridyltransferase is preferred.

Results

- weak + ve - send fresh urine to confirm.
- clear + ve - sent for chromatography to identify substance present (may not be sugar).

Causes of positive results:

Substance present	Causes
• glucose	- diabetes mellitus (the commonest) • iatrogenic • renal tubulopathy
• galactose	- galactosaemia • liver dysfunction • prem. babies & normals on high milk intake
• lactose	- primary & secondary lactase def. • late pregnancy • lactation
• fructose	- essential fructosuria • hereditary fructose intolerance (seen after weaning)
• pentose	- essential pentosuria (xylose) • alimentary pentosuria after cherries ++ & grapes ++
• homogentisic acid	- alkaptonuria (darkens on oxidising in air).
• glucuronates	- glucuronidated drugs eg. salicylate metabolites (see below)
• urate	- in health if urine is very conc.
• creatinine	- in health if urine is very conc.
• salicyluric acid	- an aspirin metabolite

renin 0d 4mL

Sent

See ACE, aldosterone, Cg A, comments, cortisol, HIAA, K, Na, 17-OHP & VMA.

Adult recumbent	0.5 - 2.2 nmol/h/L
.. ambulant	1.2 - 4.4
University College London Hospital	

- **Needs its own sample**,
- **only aldosterone assay can share**.
- Measure **aldosterone** to ↑ diagnostic performance.

Use **Ix of:**

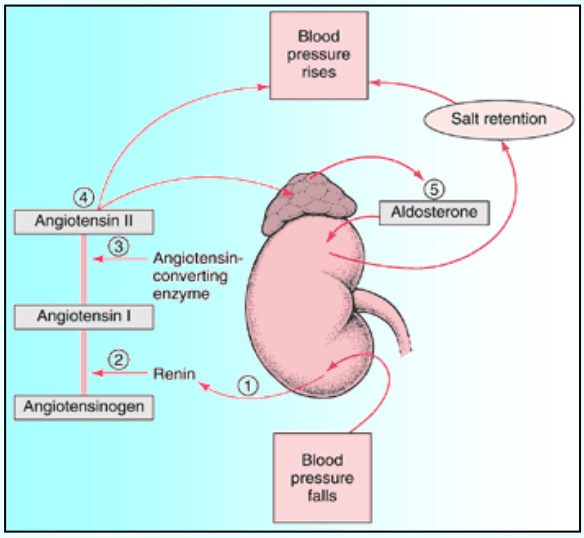
- ↑ **BP**, especially in youth or if resistant to control at any age.
- ↓ **BP**
- ↑ **K** after exclusion of common causes (see serum potassium)
- ↓ **K** .. ..
- **Monitoring CAH** (congenital adrenal hyperplasia) therapy. Renin (*without aldosterone)* assay is enough.

Test

- **Renin enzyme activity** is measured.
- **Give drug details** – antihypertensive therapy affects renin angiotensin-aldosterone physiology & can give results resembling those caused by disease.
- Renin is more **stable in EDTA** than clotted blood. However, it is best to have the **blood taken in hospital** so that it is received without possibility of degradation.
- **Drop-in phlebotomy clinics** are available at East Surrey & Crawley Hospitals.
- ***Ideally*** take samples:

- before patient even sits up in bed in the morning
- & 30 min. after getting up.

- Sampling after **30 min. rest in a chair** is usually sufficient to exclude hypo & hyperaldosteronism.



Background

- Renin is secreted by the **juxtaglomerular cells** in the media of the **afferent** (going-to) glomerular **arteriole**, in response to ↓ **ECF vol.**, ↓ **BP**, ↑ **sympathetic** nerve activity & ↑ **plasma catecholamines**.
- In contact with the exterior of the arteriole is the **macula densa**, modified tubular cells at the start of the **DCT**.
- Together they form the **juxtaglomerular apparatus**.
- Renin is a **protease** related to pepsin, which cleaves the inactive protein **angiotensinogen** (452aa, secreted by the liver) to release the decapeptide **angiotensin I**, the precursor to **angiotensin II** - a vasoconstrictor & stimulator of aldosterone secretion.
- For reasons unclear, 90% of secreted renin is the inactive precursor, **prorenin**, which emerges constitutively ie. it is not stored, regulation is slow (ca. 2h) & it is at the level of transcription & translation. See chromogranin A. Only the *ca.* 10% of prorenin in the regulated secretory pathway is converted in **storage vesicles** to the bioactive renin we are familiar with. This form in plasma is usually **measured by enzymology** & is termed "**renin activity**".
- The physiological connection between renin & aldosterone enables changes in one to confirm changes in the other ie. expression of their levels as a **ratio** gives a **more sensitive** marker of mineralocorticoid excess than either alone.

Causes of ↑

- sodium depletion
- hypovolaemia
- renal arterial stenosis
- diuretics
- standing/sitting up
- hepatic cirrhosis
- response to ↓ BP
- blood loss
- heart failure

Causes of ↓

- mineralocorticoid excess eg. Conn's, liquorice
- hyporeninaemic hypoaldosteronism
- high sodium intake
- renin inhibitors eg. Aliskiren

S

salicylate 2d 1.5mL

Daily

Therapeutic	< 300 mg/L
Toxic	> 300
Roche Cobas SALI method sheet V4	

See anion-gap, ethanol, ethylene glycol, lactate & paracetamol.

Use

- Ix of **OD**.

Background

- Aspirin (**acetylsalicylic acid**) is absorbed in minutes in the **duodenum** & **stomach** (directly via gastric mucosa).
- **Esterases** in the gut mucosa & liver rapidly hydrolyse it to **salicylic acid**.
- Further, mainly hepatic catabolism & **glucuronidation** give serum total salicylate levels a half-life of **2.4– 4.5 h**
- The **kidney excretes**:

- glucuronide conjugates
- free salicylic acid &
- free salicyluric acid

- **Urine acidity** ↑ tubular reabsorption of free salicylic acid.
- ie. **urine alkalinisation** ↑ excretion.
- Clinical effects of aspirin are due to inhibition of the enzyme ***cyclo-oxygenase*** & the resulting ↓ **prostaglandin (PG) synthesis**:

- ↓ **pain**: PGs stimulate & sensitise pain receptors
- ↓ **platelet aggregation**: Thromboxane A2 in platelets promotes aggregation & activation
- ↓ **pyrexia**: PGE1in the brain stimulates ↑ body temp.

selenium (Se) 3d 3mL
See acute phase, MRI & zinc.

Sent

0 - 16y	0.44 - 1.43 umol/L
Adult	0.89 - 1.65
Royal Surrey County Hospital	

Use

- Ix Se deficiency.
- **A less than ideal measure** because factors other than deficiency ↓ results more often (see below).

Background

- Nearly 30 proteins complex with Se (**selenoproteins**) & critically require it for their functions in:

- thyroid physiology
- antioxidant defence
- immune function eg. - *iodothyronine deiodinase*
- *glutathione peroxidase*
- *selenoprotein-P* (the most abundant selenoprotein in plasma).

- Good **sources** of Se are: brazil nuts • seafood • kidneys • meat • American rice & wheat.
- Efficiently absorbed in the **duodenum** (50 – 80% of dietary Se), organic better than inorganic.
- **Neonates** have 40-70% of the maternal plasma Se conc. (less in premature babies)

- ↓ further, especially with formula-milk feeding
- by age 4 – 6m this decline ceases
- plasma Se only reaches **adult values in late teens**.

- ↓ with the **acute phase response** due to **redistribution NOT deficiency** eg. in ICU patients mean is 40 – 60% below normal ie. **inflammation commonly co-exists** with predisposition to deficiency.
- **Illnesses** have been linked to **dietary** Se deficiency with benefit from Se supplements eg. cancer, cardiovascular disease & neuropsychiatric states such as depression
- **However**, in areas with Se-poor soil, the clinical **effects of deficiency are inconsistent** & reflect additional factors eg. in Chinese people, **cardiomyopathy** is reported, while in Tibet & central Africa, **cretinism** is seen. Both respond to Se supplements.
- In the UK, **clinically apparent Se deficiency is rare** eg. muscle weakness & pain.
- **Summary**: published works support the use of Se **supplements**, but plasma conc is a **weak guide to their use**.

Causes of ↓

- acute phase response
- TPN
- malabsorption
- extreme diets
- old age
- endemic deficiency
- anorexia
- childhood growth
- neonates
- pregnancy .

SHBG (Sex Hormone Binding Globulin) 3d 1.5mL

Daily

See free androgen index & testosterone.

Use

- Clarification of the **significance of total testosterone** results.
- **Added by the lab.** to testosterone results, as needed**.**
- Ix functional effect of thyroid hormone resistance.

Women	20 - 49 y	32.4 - 128 nmol/L
	≥ 50	27.1 - 128
Men	20 - 49	18.3 - 54.1
	≥ 50	20.6 - 76.7
Roche Cobas SHBG sheet V11 (5th-95th cent)		

Background

- A **95kDa glycoprotein** homodimer.
- **Binds** & **transports testosterone** & **oestradiol** in plasma.
- Albumin binds sex steroids too, but 1% of affinity of SHBG.
- Most SHBG is synthesised in the **liver** with a little from the **testes, placenta, vagina** & even the **brain.**
- Only unbound testosterone is bioactive. ↓ **SHBG** causes ↑ **androgenic effect** of testosterone**.**

- **WOMEN**:
  SHBG is assayed if total testosterone is > 1.0 nmol/L in order to calculate the free androgen index. This estimates the free testosterone level in case the total level in this range could be misleading due to variant SHBG expression. SHBG assay does not alter the interpretation of serum total testosterone results ≤ 1.0 nmol/L.

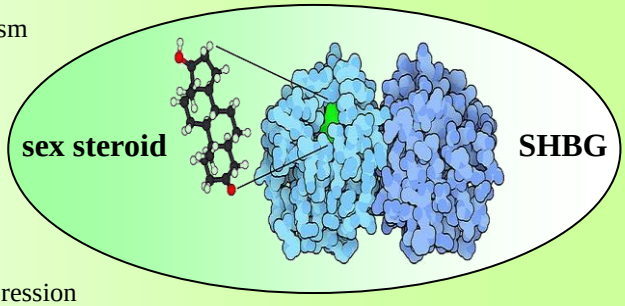
MEN:
The significance of serum total testosterone results at the ref. range limits, can be altered by variant expression of SHBG ie. SHBG is automatically assayed if total testosterone is either < 14 nmol/L or above the upper ref. limit.

Causes of ↑

- anticonvulsants
- old age in men
- oestrogens eg. OCP & pregnancy
- variant ↑ expression
- hyperthyroidism
- liver cirrhosis

Causes of ↓

- PCOS
- androgens
- hyperinsulinaemia
- hypothyroidism
- hirsutism
- obesity
- ↑ IGF1
- variant ↓ expression



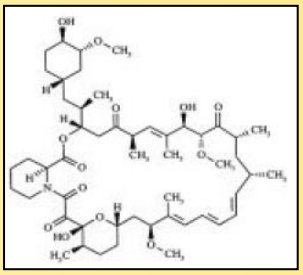
sirolimus (rapamycin) 3d 2mL

Sent

See carbamazepine, cyclosporin, phenobarb., tacrolimus & TDM.

- **Pre-dose sample.**

Depends on use
Harefield Hospital



Use • Dose-optimisation.

Background

- From the soil bacterium ***Streptomyces*** *hygroscopicus*.
- Used mostly to **control rejection** of transplanted organs.
- But, its **antiproliferative** effect is exploited elsewhere eg.
  - addition to **coronary stents** to prevent restenosis after balloon angioplasty.
  - to treat tumours of **tuberous sclerosis** & **cancer**.
- Binds cytosolic **immunophysin** in lymphocytes, like cyclosporin & tacrolimus do. **Unlike** them, the sirolimus immunophysin complex **targets mTOR** (mammalian Target of Rapamycin) instead of calcineurin.
- ie. sirolimus has different pharmacology:
  - **less nephrotoxic**
  - blocks B & T cell activation by **inhibiting** the ***response*** **to IL-2** rather than by blocking IL-2 *production*
  - it **inhibits B cell maturation to plasma cells**, thus lowering immunoglobulin production.
- Thus, sirolimus can **synergise** with cyclosporin & tacrolimus.
- Eliminated mainly by **hepatic** & **intestinal mucosal metabolism** by the microsomal enzyme CYP3A4.
- **Minimal dependence on renal excretion**.

Causes of ↑

- Hepatic impairment
- Inhibitors of CYP3A4 eg.
  - grapefruit juice
  - ketoconazole
  - erythromycin

Causes of ↓

- Inducers of CYP3A4 eg.
  - carbamazepine
  - phenytoin
  - phenobarbitone

Biochemical side effects of sirolimus:

- ↑ or ↓ K
- ↑ cholesterol
- ↑ Tg
- ↑ creatinine
- ↓ PO_4

sodium (serum) 3d See profiles

See aldosterone, anion gap, comments, creatinine, eGFR, K, **MRI**, Na (U conc), osmo (serum), osmo (U), PSA, renin & urea.

gold

Daily

136 – 145 mmol/L

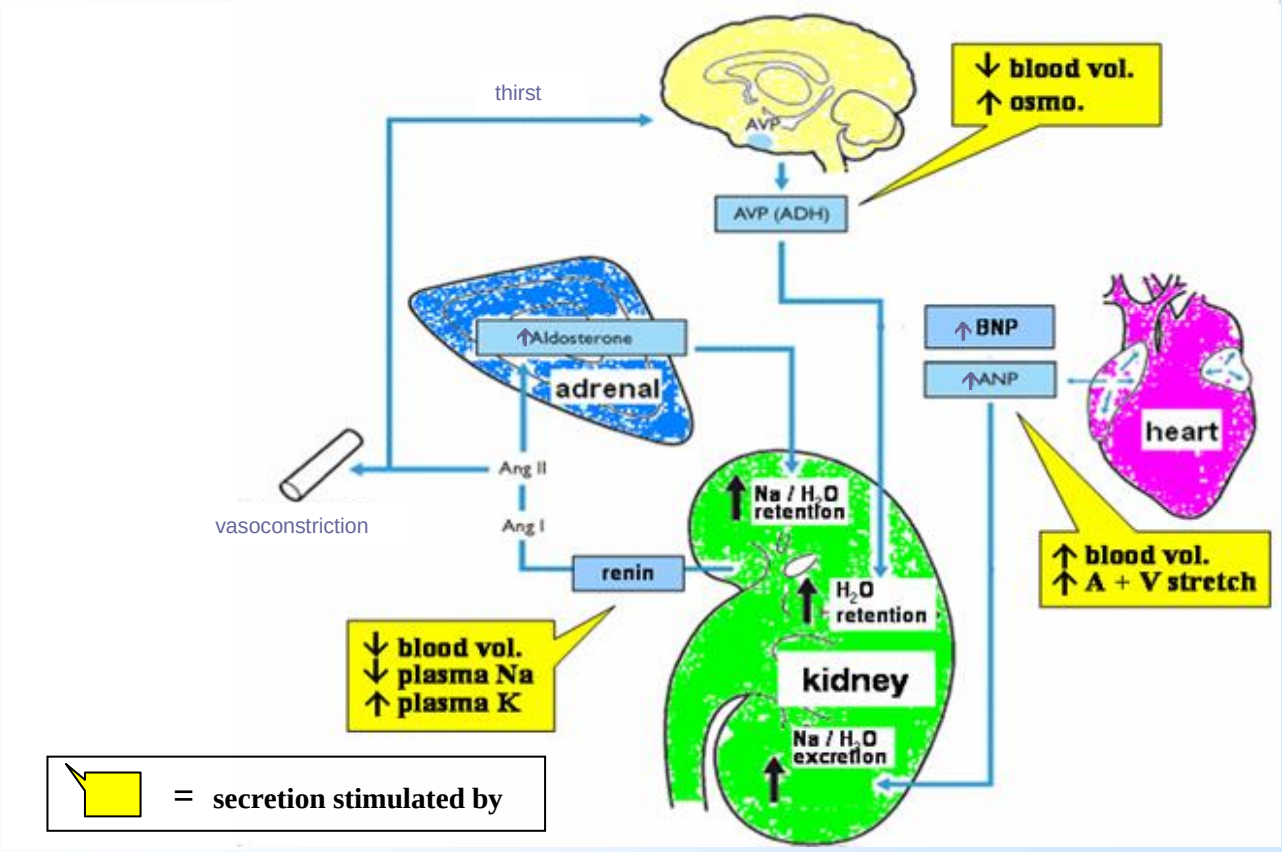
Roche Cobas ISE indirect Na-K-Cl Gen.2 V7

• **See the useful advice in the Hospital Medical Handbook.**

Background

• The physiology of salt, water & ECF volume regulation enables most clinical situations of hyper & hyponatraemia to be understood & managed in a few steps:

Step	Actions and responses
1	Note drugs, fluid & electrolyte balance & diagnoses, which have a bearing on Na & H_2O.
2	Determine the **ECF vol. by examinination** eg. oedema & JVP. Na conc. is only the Na/H_2O ratio & says little about the ECF vol., which is the main determinant of Na & H_2O physiology.
3	Note any specific physiological consequences of the diagnosis eg. in oedema, although aldosterone is hypersecreted & might cause hypernatraemia, ADH is hypersecreted too, which leads to hyponatraemia.
4	Put it all together into a prediction of the state of extra-cellular fluid (ECF) regulatory physiology ie. ask, "what do I think the physiology is aiming to do?"
5	• Measure osmo. & Na conc. in spot urine. • Is the kidney conserving Na (urine Na <30 mmol/L) & water (osmo. > 400 mmol/kg or losing them? • Do the results confirm your physiological predictions? • If the kidneys are conserving Na, where else is it being lost? • Is Na intake adequate? • Remember, natriuresis is triggered by ↑ ECF vol. to prevent overload eg. in SIADH & polydipsia, even in the presence of hyponatraemia. Thus, high urine Na conc. does not automatically mean renal disease. Renal loss causes ↓ ECF vol. & can be 2ndy too eg. diuretics & hypoaldosteronism.



The physiology of H_2O, Na & vol. regulation enables clinical observations of ECF vol. to be integrated & the response to abnormalities of Na & H_2O (especially hypo & hypernatraemia) to be hypothesised & tested. This aids understanding & appropriate management eg. fluid restriction rather than salt for hyponatraemia due to SIADH.

sodium (sweat) 3d

Chloride is now the preferred analyte.

See chloride (sweat)

sodium (urine conc.) 3d

Daily

See comments, Na (serum) & osmo (urine).

40 - 220 mmol/L
Roche Cobas ISE Na-K-Cl Gen.2

Use

- Ix. of hyponatraemia.

Background

- Urine sodium concentration is highly dependent on **intake** & **physiology**.
- Interpret urine Na only after clinically determining the **ECF vol.** & the likely physiological state of the patient (see serum sodium) eg. if the patient should be conserving Na, urine Na conc. is likely to be <30 mmol/L. In SIADH, urine Na can be > 30mmol/L despite hyponatraemia, because of natriuresis to limit ECF vol. expansion.

somatostatin 0d 6mL + **aprotinin** Contact lab.

Sent

See gut hormone profile.

- **Keep samples on ice.**

< 150 pmol/L
Charing Cross Hospital

squamous cell carcinoma antigen (SCC Ag)

See CYFRA 21-1, CEA, CA12.5, CA15.3 & CA19.9.

- **Replaced by CYRA 21-1**

Use

- Monitoring **squamous carcinoma** (cervix, bronchus, oesophagus, head & neck) & the response to therapy.

sugar chromatography 1d fresh urine (5 mL)  or faeces  Sent
(syn. oligosaccharides). See Gal-1-PUT, reducing substances (faeces) & reducing substances (urine).

Refs & comment sent with result
Great Ormond Street Hospital

- **Needs its own specimen.**
- **Normal diet.** Exclusion diets can cause **false –ves** eg. if lactose (milk sugar) is avoided in galactosaemia.
- Urine or faeces are tested for reducing substances & only sent for sugar chromatography if +ve.

Use

- **Identification of the cause of a positive reducing substances result** in urine or faeces.

steroid profile 3d Ideally 24h urine  Sent
See 11-deoxycortisol, 17-OHP & virilisation.

Refs & comment sent with result
University College London Hospital

Use

Babies & children only 20mL

- Ix babies born with a **disorder of sexual development** (DSD).
- enables congenital adrenal hyperplasia (CAH) to be diagnosed asap for the benefit of baby & parents.
- Ix a **steroid secreting neoplasm** of the adrenal cortex or ovary.

Background

- **Chromatography/mass spectrometry** can measure dozens of steroids & their metabolites in urine.
- It is the **gold standard for investigating disorders of adrenocortical steroid synthesis**, especially in the first week of life when the immaturity of the neonatal adrenal cortex & liver, combined with the remnants of placental steroidogenesis, make the quality & quantity of circulating steroids, even in health, very different from later in childhood & adulthood.
- Measurements of single analytes eg. 17-hydroxyprogesterone, can be misleading within 3 days of birth because of interference from normal, but transiently elevated substances eg. 17-hydroxypregnenolone.

stone analysis 3d ideally intact but grit will do Sent 
See Ca, Ca (24h urine), cystine, oxalate, PO_4, PO_4 (24h urine), uric acid & UA (urine).

Refs & comment sent with result
Univ. College London Hosp.

Use

- Ix **nephrolithiasis**

Background

- Stone analysis **identifies organic stones well** (urate, oxalate, cystine). They are commoner in children & young adults compared with the older patients.
- Ca, PO_4, oxalate & ammonium are non-specifically & commonly present in many stones ie. merely finding them has little value & blood-tests of Ca, PO_4, U+E, urate & PTH are more useful.

sulphonylurea 0d 2mL

See C-peptide, glucose & insulin.

- **Take blood for plasma glucose too.**

PLUS

Sent

Absent
Royal Surrey County Hospital

Use

- Ix of **hypoglycaemia** with **C-peptide** & **insulin** hypersecretion, possibly due to sulphonylurea use.
- Assay in patients on normal treatment, is unnecessary, even if they are hypoglycaemic.

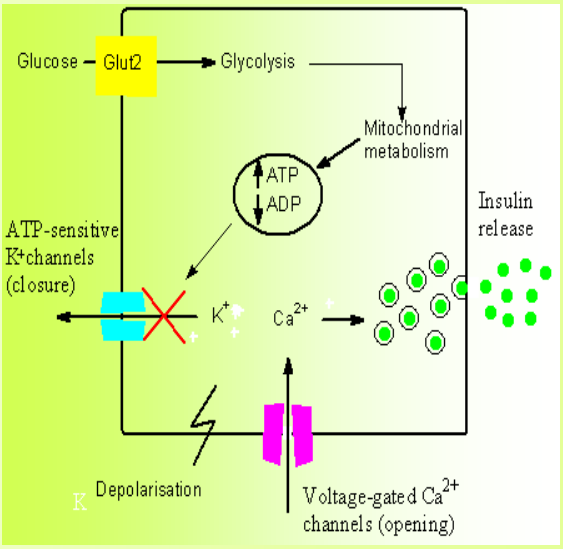
Background

- Sulphonylurea drugs stimulate insulin release from beta cells, by binding to the sulphonylurea receptor (**SUR**) proteins of the **ATP-dependent potassium channel** (K_{ATP}) in cell membrane.

- **K_{ATP} is a complex** of 4 SUR protein subunits & 4 inward rectifier potassium ion channel (Kir6) subunits.

- Binding of **sulphonylurea closes the K_{ATP}** channel, like ↑ intracellular ATP from ↑ plasma glucose would normally do. **This causes:**

- beta cell **depolarization**
- opening of voltage gated Ca channels
- ↑ cytosolic Ca conc., which leads to
- migration of insulin-storage vesicles to the plasma membrane where they fuse with it & discharge their contents.



Interpretation

- Hypoglycaemia with ↑ C-peptide & ↑ insulin at the time, indicates autonomous **endogenous** insulin secretion eg. due to insulinoma or sulphonylurea drugs. **Exogenous insulin** is C-peptide-free & the hyperinsulinaemia it causes is unaccompanied by ↑ serum C-peptide, because β cells (the only source) are suppressed by hypoglycaemia.

T

tacrolimus (FK506) 3d 2mL

Sent

Ref. depends on use. (ug/L)
Royal Sussex University Hospital & other sites if requested.

See cyclosporin, sirolimus & therapeutic drug monitoring,

- **Pre-dose sample.**

Use

- Optimisation of tacrolimus Px

Background

- This immunosuppressant from the bacterium *Streptomyces tsukubaensis* is used:

- to control rejection in **organ transplantation**
- in the management of **inflammatory diseases** eg. IBD, atopic eczema & vitiligo.

- It binds the cytosolic protein **immunophilin** in lymphocytes.
- The complex then acts on **calcineurin** to:

- inhibit T lymphocyte function
- ↓ production of IL-2 (IL-2 would normally activate B & T lymphocytes).

- Tacrolimus is **metabolised in the liver** by CYP3A4 & excreted mostly in **bile.**

Causes of ↑

- **Grapefruit** juice & **antifungals** eg. fluconazole, compete for CYP3A4 & **impede elimination**
- Sampling too soon ie. in the distribution phase of the drug.

Biochemical side effects in serum:

- ↑ creatinine
- ↑ urea
- ↑ K
- ↑ glucose
- ↑ cholesterol
- ↑ LFT
- ↓ Mg

tau protein (β2 transferrin) 3d 2mL of fluid

Sent

Negative
Sheffield Protein Reference Unit

See carbohydrate deficient transferrin (CDT) & protein electrophoresis.

Use

- To answer: **is this watery discharge** from the ear/nose/wound/drain, **CSF?**

Background

- **Transferrin in CSF is less glycosylated** than the familiar plasma glycoprotein form β1 transferrin.
- This **asialylated transferrin** is termed **Tau protein** & runs in the **β2** region on electrophoresis of CSF proteins.
- In contrast, circulating transferrin runs in the **β1** band.
- Tau protein **enables CSF to be distinguished from tissue/serous fluid**, but mixtures can give indistinct results.

testosterone 2d 1.5mL

Daily

See androstenedione, DHEAS, DHT, FAI, FSH, 17-OHP, LH, LHRH test, **MRI**, oestradiol & SHBG.

Women	20 - 49y	0.29 - 1.67 nmol/L
	≥ 50y	0.10 - 1.42
Men	20 - 49y	8.64 - 29.0
	≥ 50y	6.68 - 25.7

Roche Cobas Testosterone II mthd sheet V7 (5^{th} – 95^{th} centiles)

Tanner stage	**Girls** nmol/L	**Boys** nmol/L
1	< 0.1 – 0.2	< 0.1
2	< 0.1 – 0.4	< 0.1 – 15.0
3	< 0.1 – 0.8	2.3 – 27.0
4	< 0.1 – 0.9	6.2 – 26.5
5	0.2 – 1.3	6.5 – 30.6

Roche Cobas Testosterone II method sheet V7 (5^{th} – 95^{th} centiles)

MALES

DIAGRAM. See LH

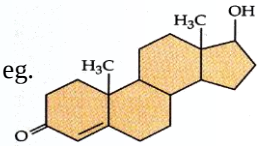
- Luteinizing hormone (**LH**) secreted by the gonadotropes of the anterior pituitary, stimulates testicular **Leydig cells** to secrete testosterone which feeds back to the hypothalamus & pituitary to inhibit LH release.
- A little testosterone is secreted by the **zona reticularis** of the adrenal cortex.
- Testosterone targets eg. external genitalia, are insufficiently sensitive to respond to testosterone without its conversion to the more potent androgen, **dihydrotestosterone**, by the intracellular enzyme **5∝-reductase**. The crucial importance of this step is illustrated by cases of 5∝-reductase **deficiency** in XY males, which may only come to medical attention because of amenorrhoea in apparently normal girls.
- Serum testosterone levels **peak in early morning** & are ↑ by **exercise**.
- Sex hormone binding globulin (SHBG) binds testosterone in both sexes & ↓ levels of the bioactive, free-form.

Causes of ↓

- ↓ SHBG
- **Primary** hypogonadism (↑ FSH & ↑ LH) eg.
  - Kleinfelter's syndrome.
  - Haemochromatosis.
  - Orchitis.
- **Secondary** hypogonadism (hypogonadotropic hypogonadism, ↓ or low-normal FSH+LH) eg.
  - Hypopituitarism.
  - LHRH analogues eg. buserelin.
  - Prader-Willi syndrome.

Causes of ↑

- ↑ SHBG. See SHBG
- Testo. HRT (↓LH, ↓FSH)
- **"body-builder"** – (↓LH, ↓FSH, ↑oestradiol...



FEMALES

DIAGRAM. See LH

- In women of reproductive age, testosterone is secreted by **theca cells of ovarian follicles** (see oestradiol), the **placenta** & the zona reticularis of the **adrenal cortex**.
- Additionally, **weak androgens** eg. DHEAS & androstenedione secreted by the adrenal cortex & ovaries, are **converted to testosterone** (aromatised) in peripheral tissues eg. **adipose tissue**.
- Despite its low levels in women, testosterone contributes to psychological well-being, prevention of osteoporosis & maintenance of muscle mass.
- The impact of female total testosterone levels above 1.0 nmol/L (but still within the reference range) is ↑ by ↓ **serum SHBG**. ↓ in this causes ↓ bound inactive form & ↑ levels of the bioactive, free hormone.
- In women with menstrual or infertility problems, measure serum **DHAS** & **testosterone** (ideally in the **follicular phase** when false + ves are less frequent) because clinical evidence is an insensitive marker of significant androgen excess, which may have an impact on eg. fertility & menstruation.
- **NB.** The absence of *clinical* evidence of androgen excess does not exclude the presence of PCOS (Rotterdam criteria).

Causes of ↑

- PCOS
- Obesity
- Congenital adrenal hyperplasia (CAH)
- Adrenal & ovarian tumours
- Cushing's disease
- ↑ SHBG

Causes of ↓

- Assay-**precision is inadequate to identify deficiency**.
- A syndrome of deficiency defined by results is thus unclear.
- The use of testo. HRT should be **guided clinically**, NOT by results.

theophylline 3d 1.5mL

Daily

10 - 20 mg/L
Roche Cobas THEO2 method sheet V3

See caffeine & therapeutic drug monitoring.

Use

- To aid: - effective treatment with minimal side effects
  - management of toxicity
  - confirmation of compliance if clinical effect is lost or difficult to achieve.

Background

- This drug, like caffeine, inhibits **phospodiesterase**, the enzyme that degrades cAMP. Thus, intracellular cAMP levels ↑ & cause CNS stimulation, bronchial smooth muscle relaxation & bronchodilation.
- **Less toxic bronchodilators have replaced its use in adults** but it has use in treating premature **neonates with apnoea**. Even here, it has been substantially replaced by less toxic drugs which are easier to use eg. caffeine.
- Clearance by **hepatic metabolism** (35% is converted to caffeine) is highly **variable** eg. neonatal half-life = 24h, older children = 4h & smokers < non-smokers (see TDM). The clinical effect of a given dose, is unpredictable & serum levels correlate better. Hence the need for drug levels.
- **Do not sample < 5 half-lives after dosage change or starting** ie. before equilibrium. See TDM for details. In overdose situations requiring haemodialysis, levels may need to be assayed as often as 4 hrly

Causes of ↑ • heart failure • hepatic cirrhosis • hepatitis • liver failure

Causes of ↓ • smokers • alcoholism • hepatic enzyme inducing drugs

thiamine (vitamin B1) 0d 2mL

Sent

See vit B1
Rotherham Hospital

See folate, vit. B1, vit. B6, vit. B12 & vit. D.

- **Fasting needed.**
- **Protect from light** with aluminium foil.
- **Ideally**, take the sample at ESH or Crawley Hospital to minimise delay getting to the lab.

Background

- Thiamine is metabolised to the active form, **thiamine pyrophosphate** (TPP), a cofactor for key enzymes involved in major **oxidative decarboxylation** processes eg.

- **pyruvate → acetyl CoA** by *pyruvate dehydrogenase* at entry to the TCA cycle
- **α-oxoglutarate → succinylCoA** by *oxoglutarate dehydrogenase* within the TCA cycle
- **branched chain a.a. metabolism** (leucine, isoleucine & valine) by *branched chain keto-acid dehydrogenase*.

- Thus, substantial oral or iv. intake of **carbohydrate can acutely worsen** the effects of thiamine deficiency because of the need for it in carbohydrate metabolism.
- TPP is also a cofactor for **transketolase** in the **pentose phosphate pathway** which synthesises:

- **pentoses** eg. ribose
- **NADPH** for fatty acid synthesis & reduction of glutathione to restore its antioxidant capacity.

- **Direct thiamine assay** is now preferred to **transketolase** as a marker of thiamine deficiency.
- Deficiency causes **beri-beri**. 2 types:

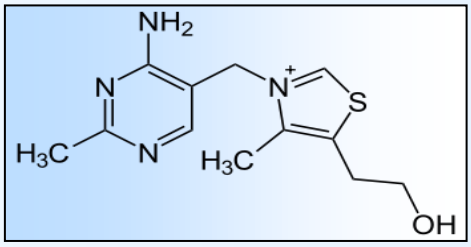
- **dry** beri-beri (sensorimotor polyneuropathy, encephalopathy & Korsakoff syn.)
- **wet** beri-beri (high output cardiac failure with peripheral & pulmonary oedema).

Why the dichotomy? Deficiency of additional dietary factors & genetic variation of the affected enzymes.

- **SOURCES**: widespread but especially wheat germ, oatmeal & yeast.

Causes of ↓

- alcoholism
- poor diet eg. anorexia nervosa
- polished rice (B1is in the husk, which is removed)
- malabsorption
- short gut
- bariatric surgery



thioguanine nucleotides 3d 1mL
See DYPD, MMPN, TDM, TPMT & uric acid.

Sent

235 – 450 pmol/8x10^8 rbcs
City Hospital, Birmingham

Use • Optimisation of azathioprine & mercaptopurine Px. TGN can be misleading in MMPN toxicity, see below

Background

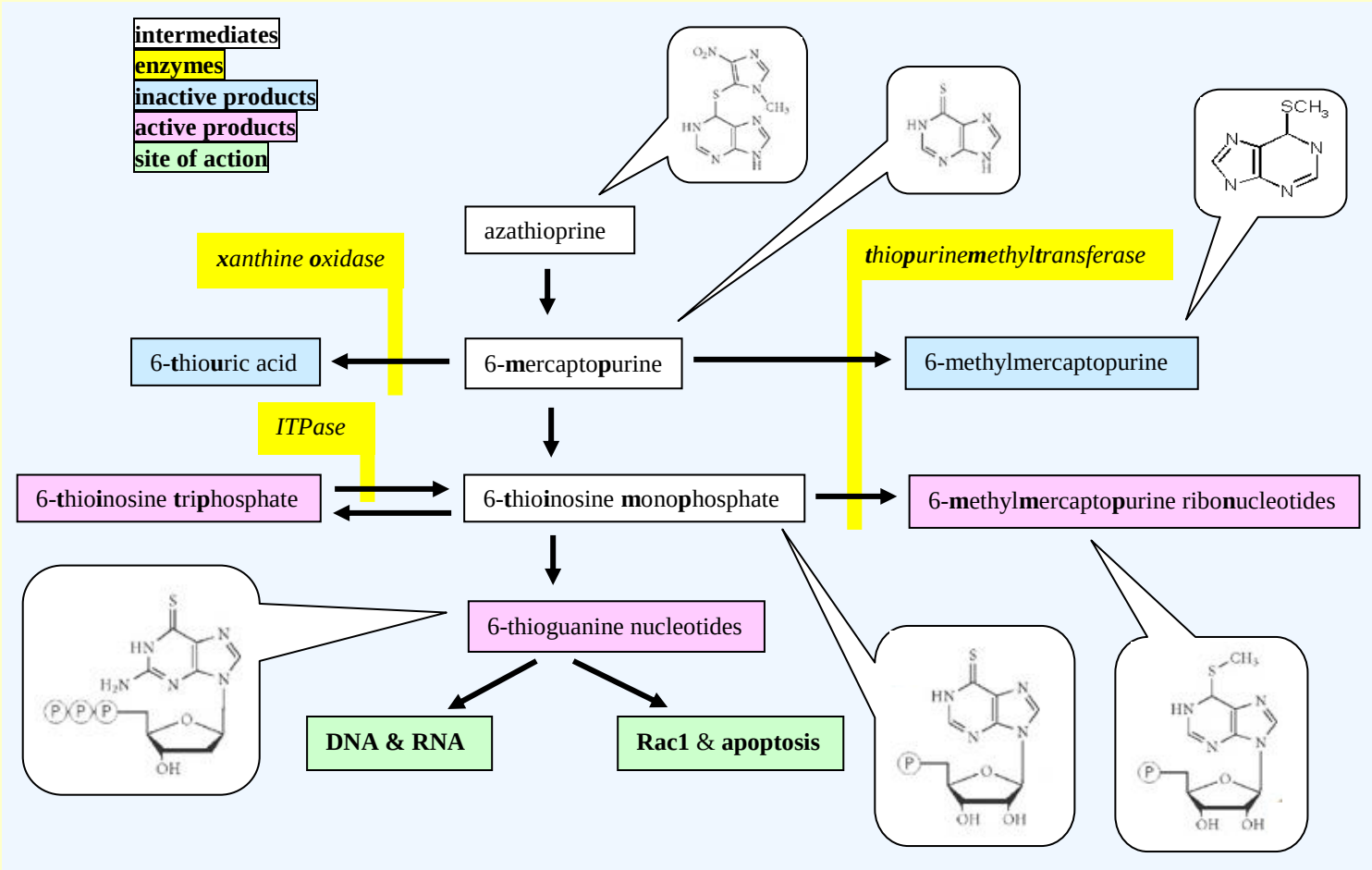
- **Azathioprine** (AZA) is a purine analogue immunosuppresant used to 1) treat autoimmune conditions eg. IBD, eczema & Rh disease & 2) control rejection of transplantated organs.
- It is rapidly & non-enzymatically converted to **6-mercaptopurine** (6MP) which then enters a complex metabolic pathway with alternative active & inactive products, the levels of which are influenced by 1) allelic variation of enzymes, 2) other drugs & 3) unknown factors.
- **6-thioguanine nucleotide** (TGN) is the principal product of AZA (& thioguanine & mercaptopurine) & acts by binding instead of GTP to **Rac1** (a signalling molecule inside T cells) which results in **↑ T cell apoptosis**.
- A nucleotide is composed of a purine group & a ribose or deoxyribose sugar bearing a phosphate group.
- ***Thiopurine methyltransferase*** (TPMT) activity is a major determinant of toxicity. TPMT normally inactivates 6MP. Deficiency allows more 6MP to become TGN, resulting in toxic levels at standard AZA doses.

Causes of ↑

- Px dose**,** xanthine oxidase inhibitors (eg. allopurinol), ↓ 6MP inactivation & ↑ TGN synthesis.

Causes of ↓

- Px dose
- Some patients process 6MP to large amounts of the active substance methylmercaptopurine ribonucleotide (**6-MMPN**) even if TPMT activity is normal, causing ↓TGN & ↑MMPN levels. **Liver failure** can result, especially if the AZA dose is ↑ to raise the TGN level.
- Deficiency of the enzyme ***inosine triphosphatase*** (ITPase), which normally inactivates thioinosine triphosphate (**TITP**) & returns it for synthesis of TGN, causes ↓ TGN & toxic ↑ of TITP.



thiopurine methyltransferase (TPMT) 3d 2mL

Sent

See DYPD, MMPN & thioguanine nucleotides.

- **Blood transfusion in previous 3m may mask a low result.**

AFTER 1/8/2010, references & units		
Category	**Activity**	**Prevalence**
Deficient	< 10 mU/L	0.3%
Low	20 – 67	11%
Normal	68 – 150	89%
High	> 150	
City Hospital, Birmingham		

Use

- To detect ↓ clearance & ↑ **risk of toxicity with thiopurine drugs** eg. azathioprine (**AZA**) & 6-mercaptopurine.
- TPMT activity **MUST be measured before** giving these drugs.
- TPMT activity does not need to be measured more than once.

Background

BEFORE 1/8/2010, references & units		
Category	**Activity**	**Prevalence**
Deficient	≤ 5 nmol/g Hb /h	0.3%
Low	6 – 34	11%
Normal	35 – 79	89%
High	≥ 80	
City Hospital, Birmingham		

- TPMT is **a key enzyme in the inactivation of azathioprine** (AZA), 6-mercaptopurine & 6-thioguanine.
- These drugs are widely used to treat autoimmunity, graft rejection & leukaemia.
- TPMT **methylates 6-mercaptopurine** (6MP), which is **formed non-enzymatically from AZA**. The more 6MP that is inactivated, the less that can become active product, 6-thioguanine nucleotide (TGN). DIAGRAM of pathway.
- Alleles which ↓ **TPMT activity,** cause ↑ risk of **severe myelotoxicity** with AZA at standard doses, because less is inactivated.
- V. rare alleles have ↑ **activity**, resulting in ↑ clearance & **need to ↑ dose** to prevent **treatment failure**.
- **TPMT genotyping** (testing DNA for mutations) is done to 1) confirm low activity, 2) if thiopurine must be given before recently transfused blood can be cleared & 3) toxicity occurs despite apparently adequate activity.

Causes of ↓

- allelic variation

Causes of ↑

- allelic variation
- TPMT enzyme induction by AZA & 6-mercatopurine
- Blood transfusion within 90 days of the sample, can increase activity from “deficient” to “low”.

thyroglobulin 3d 2mL

See calcitonin, FT3, FT4 & TSH.

Sent

1.4 - 78 ug/L
Sheffield Protein Reference Unit

Antithyroglobulin Ab (ATG)
≤ 115 kIU/L
Sheffield Protein Reference Unit

Use

- Monitoring **follicular** & **papillary** thyroid carcinoma after total thyroidectomy.

Background

- A 660 kDa **iodinated glycoprotein** unique to the thyroid gland.
- **Proteolysis** of thyroglobulin in thyroid follicles, releases mono & di-iodinated tyrosine residues**:**

- thyroxine (**T4**) is formed from 2 di-iodotyrosines.
- tri-iodothyronine (**T3**) is formed from 1 mono- & 1 di-iodotyrosine. Diagram of T3 & T4 molecules.

- **After total thyroidectomy or ablation**, its tissue specificity means that **it should be undetectable** if there is no thyroid tissue anywhere else in the body.
- Serum thyroglobulin is **not for diagnosis of neoplasia before biopsy** for the obvious reason that it is present in health & there is significant overlap between pathological & normal levels.
- **Antibodies to thyroglobulin** in the patient sample can interfere to give **falsely low results**.
  This is much less likely with modern immunometric (2-site) assays than with immunoassays (1-site).

Causes of ↑

- **Physiologically** in healthy **neonates** & the 3rd trimester of **pregnancy**.
- Follicular & papillary **thyroid carcinoma** in the absence of normal thyroid tissue.

tiagabine 3d 3mL

No gel

Sent

See therapeutic drug testing.

- **Pre-dose sample**

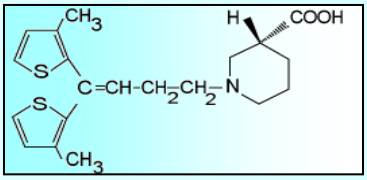
References sent with result
St. Thomas's Hospital

Use

- Ix of toxicity, compliance & when control is difficult to establish or is inexplicably lost.

Background

- **Not for routine monitoring**, poor correlation of serum level with clinical effect.
- But levels may help in Ix of compliance, toxicity & loss of or failure to establish clinical effect.
- **Inhibits** GAT1, a transporter for **reuptake of GABA** (an inhibitory neurotransmitter) from the synapse.
- For adjunctive treatment of **epilepsy**
- **95% metabolised** in the **liver** by cytochrome P450 enzyme, **CYP3A**.
- < 5% excreted unchanged in urine.
- **Half-life** = 8h, less with enzyme induction eg. by anticonvulsants.



TIBC (total iron binding capacity) 3d See profiles

See acute phase response, ferritin, folate, iron, iron satn, UIBC & vit B12.

Daily

44.8 – 76.1 umol/L
Tietz

Background

- TIBC is the maximum iron that serum proteins can bind ie. unsaturated iron binding capacity (UIBC) + serum iron
- TIBC = serum iron + UIBC
- A **marker of transferrin**.
- **Iron saturation** $\left(\frac{\text{serum iron}}{\text{serum iron + UIBC}}\right)$ is **a better marker** of iron stores than either UIBC, TIBC or iron alone.

Causes of ↑

- iron deficiency (↓ iron & ↓ iron satn.)
- hepatitis
- pregnancy
- oestrogens from any source.

Causes of ↓

- inflammation (acute phase response)
- nephrotic syndrome
- malnutrition
- malignancy
- haemochromatosis
- megaloblastic & haemolytic anaemia.

tobramycin 3d 2mL

See amikacin, gentamicin, TDM & vancomycin.

Sent

mg/L
Royal Brompton Hospital

Availability

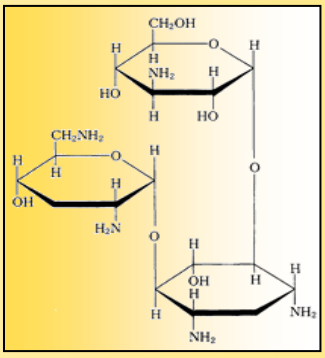
- Get samples to the lab. **by these times** for same-day results. Late samples will wait until next day.

- Mon – Fri **17.00** h
- Sat + Sun **13.00** h

Contact Microbiology for advice on interpretation & patient management.
See IPCAS on the SASH intranet for sample timing, target levels, responses to results, doses etc.

Background

- An **aminoglycoside** antibiotic, like gentamicin, which inhibits bacterial ribosome assembly & protein synthesis.
- **Toxic** to renal **tubules** & the inner **ear**.
- **Not absorbed from the gut**. ie. given iv or im
- 90% **renal elimination**.
- Plasma **half-life** in health is **2–3h**, but 5–70h if GFR ↓.
- In **neonates**, half-life is 2–11h, shorter if under 1.2 kg.



Causes of ↑

- sampling in the **distribution phase** post-dose
- ↓ GFR

Possible biochemical side-effects of tobramycin:

- ↑ serum urea & creatinine
- ↓ Ca
- ↓ Mg
- ↓ K
- ↓ Na
- ↑ AST, ALT, bilirubin, LDH.

topiramate 3d 1.5mL

Sent

5 – 20 mg/L
St. Thomas' Hospital

See therapeutic drug monitoring.

- **Pre-dose sample.**

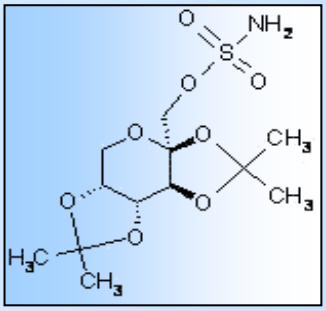
Use

- **Routine monitoring is unnecessary but assays may help in:**

- suspected **toxicity**,
- unexpected **loss of effect** or difficulty in establishing it
- **co-prescription** with drugs which ↑ topiramate clearance eg. carbamazepine

Background

- An atypical **anticonvulsant & mood-stabilizer** (structurally similar to fructose) used to treat epilepsy & bipolar disorder & to prevent migaine.
- It enhances **GABA** activated chloride channels (inhibitory signalling) & inhibits excitatory neurotransmission.
- **30%** of a dose is **metabolised in the liver**
- **70%** excreted **unchanged in urine**.
- Mixed impact on microsomal **P450** activities, it induces some & inhibits others.
- ie. **effects on other drugs** are varied.



Causes of ↓

- ↑ topiramate catabolism due to enzyme induction eg. by carbamazepine

Causes of ↑

- ↓ GFR
- Pos-dose sample ie. in the distribution phase.

Biochemical side effects

- ↑ serum phenytoin
- ↓ oestrogen (↓ OCP effectiveness)
- ↓ digoxin levels.
- metabolic acidosis from bicarbonate loss due to topiramate's weak carbonic anhydrase activity.

triglyceride 3d See profiles

See amylase, chol:HDL ratio, cholesterol (total), comments, FFA, glycolipid, HDL, LDL & **MRI**.

- **Fasting essential.**

Daily

≤ 2.3 mmol/L
Roche Cobas TRIGL method sheet V4

Background

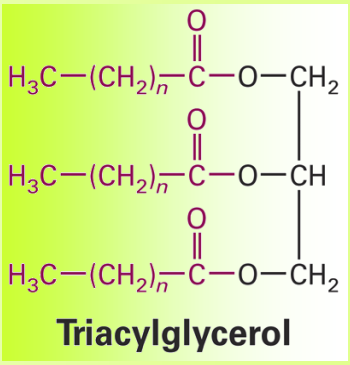
- Triglyceride (Tg, **triacylglycerol**) consists of 3 long chain (acyl) carboxylic acids (**fatty acids**) ester linked to the 3 hydroxyls of **glycerol.** See neutral lipid in glycolipid.
- Mono & diglycerides also exist.
- The acyl groups may be **unsaturated** (1 or more double bonds in the chain) or **saturated** (only single bonds).
- Tg is by far the major form of **stored energy** & supplies >80% of aerobic energy needs, especially in muscle. Glucose is for specialised use.
- Serum Tg > 4.5 mmol/L means that **LDL cannot be calculated**.
- Serum Tg > 15 mmol/L gives a high risk of **acute pancreatitis**.

Causes of ↑

- postprandially.
- **primary hyperlipidaemia** types I, IIb, III, IV & V eg. familial hypertriglyceridaemia & lipoprotein lipase deficiency. They **interact with acquired factors** eg. diabetes, ethanol or obesity ie. remedy of these is crucial.
- **secondary hyperlipidaemia**:

- obesity
- diabetes
- impaired glucose tolerance
- alcoholism
- hypothyroidism
- acute stress
- anorexia nervosa
- hepatitis
- hepatic cirrhosis
- acute & chronic pancreatitis
- extrahepatic biliary obstruction
- nephrotic syndrome
- -.CRF
- Down's syndrome

- **glycogen storage diseases** types I, III & IV



Causes of ↓

- hypo & a-β-lipoproteinaemia
- hyperthyroidism
- malnutrition
- malabsorption
- liver failure

troponin T hs (cTnThs) 1d 1.5mL

Daily

High senstivity cardiac troponin T assay.
See AST, BNP, CK, LDH & **MRI**.

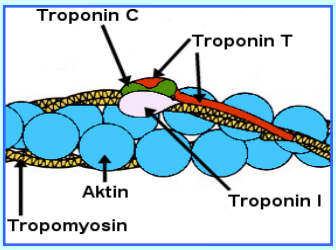
	SAMPLE TIMING	
	On admission	**and 6 h later**
Evidence of myocardial injury	**≥ 15 ng/L**	**≥ 15 ng/L**
Normal upper limit (99th centile)	14	14
C.V 10 % at:	13	13
Evidence of *acute* injury 1 or more cTnT ≥ 15ng/L **&** ↑ or ↓ as right**:**		**< 20 %** Inconsistent with acute event.
Exclusion: cTnT ≤ 14 at 0h & 6h, & consistent with clinical & risk data.		**20 – 100 %** Significant, but suggest further evaluation to distinguish acute from chronic causes of ↑ cTnT.
Beware false negs. Sample at 12h too if 6h result does not fit clinical suspicion of an acute event.		**> 100 %** Consistent with acute MI.
Roche Cobas Troponin T hs method sheet V6, Thygesen K et al 2007. NICE CG95		

Use

- **Diagnosis of acute myocardial necrosis.**
- The ↑ **sensitivity** of this cTnT assay enables smaller & earlier MI detection.
- Testing can be done **on presentation** eg. with chest pain, rather than at > 6h after symptom-onset

Timing

- Measure on **presentation** & **6 h later**.



Interpretation See above.

- **cTnT >14 ng/L** at any time is evidence of myocardial necrosis (Thygesen 2007)
- **Change** (↑ or ↓) distinguishes acute from chronic causes of ↑ cTnT.
- **Interpret all results in conjunction with clinical** & **risk data.**
- **False + & – ves** occur but the change-requirement for diagnosis of acute MI ↓ the risk of being mislead.
- Measure cTnT at **12h too** if the 0 & 6h results are inconsistent with clinical data.

Background

- Troponins C, I & T form complexes dotted along **tropomyosin** molecules which parallel actin filaments & inhibit interaction with myosin.
- **TnC** (**same in all muscles**) responds to **Ca** from the sarcolemma.
- **TnT** binds TnC & TnI to tropomyosin.
- **TnI** blocks the sites of actin-myosin interaction.
- **TnT** & **TnI have truly cardiac-specific forms**, unlike CK.
- ↑ cTnT occurs with ANY myocardial injury ie. it is **not pathology specific.**
- Serum cTnT starts rising 4 – 6h after injury, like CK, & **persists for 7 – 10d** (1 – 2d with CK).

Causes of ↑ Not just MI

- ischaemic heart disease
- aortic valvular disease
- tachy/bradyarrythmias
- CRF
- scleroderma
- burns, esp. >30%
- coronary vasculitis eg. SLE, Kawasaki dis.
- cardiac trauma incl. iatrogenic
- hypertrophic cardiomyopathy
- CVA
- haemochromatosis
- endo & pericarditis
- cardiotoxic Px
- A & C HF
- PE ++
- SAH
- amyloidosis
- myocarditis
- cocaine
- aortic dissection
- pulmonary hypertension ++
- exercise+++
- sarcoidosis
- critical illness
- defib.

tryptase 3d 4mL

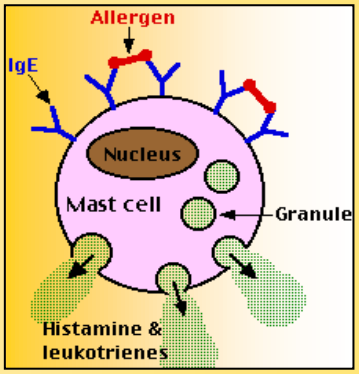
Sent

- **Send clinical & drug details** with the request.

2 – 14 ug/L
Anaphylaxis: peak is typically > 40 ug/L
Sheffield Protein Reference Unit

Use

- **Anaphylaxis** confirmation. Sample at < 1h, 3h & 24h after the episode. Even 1 sample taken in an hour or so can be very useful.
- **Mastocytosis** Ix & monitoring. Only 1 sample needed, taken anytime.



Background

- Tryptase is **stored in mast cell secretory granules** with the better known molecules, **histamine & serotonin**.
- After release by **mast cell degranulation** in **anaphylactic** & **anaphylactoid** reactions, tryptase (halflife **2.5h**) is cleared from plasma much more slowly than histamine (halflife **2.5 min**).
- ie. tryptase is a **more practicable marker of anaphylaxis than histamine**, which is difficult to measure without significant degradation.

TSH (thyroid stimulating hormone) 3d See profiles

Daily

See calcitonin, comments, drugs & TFTs, FT3, FT4, LH, Li, **MRI**, TDM, thyroglobulin & TRH test.

- **Give clinical details** eg. on T4, T3, pregnant…

Use

- The premier test of thyroid function & HRT.

Background

- High sensitivity TSH assay is the single most reliable thyroid function test (TFT), being able to reveal hypo-thyroidism & to distinguish the ↓ levels of hyper-thyroidism from normal variation.
- TSH assay is **the primary TFT** at SASH.
- FT4 assay is added to all ↑ or ↓ TSH results.
- Unlike FT3 & FT4 which are "on their way" to tissues, **TSH is a tissue response** to their impact rather than a presumption of their adequacy.

	Age	TSH mU/L
Children	0 - 3d	5.17 - 14.6
	4d - day before 2m old	0.43 - 16.1
	2m - day before 2nd b'day	0.62 - 8.05
	2y - day before 7th b'day	0.54 - 4.53
	7y - day before 12th b'day	0.66 - 4.14
	12y - 19th b'day	0.53 - 3.59
Adults	women	0.30 - 3.94
	men	0.30 - 3.18
Pregnancy	1st trimester	0.33 - 4.59
	2nd	0.35 - 4.10
	3rd	0.21 - 3.15

Roche Cobas TSH method sheet V21 & Ref. Intervals for Children & Adults Elecsys Thyroid Tests. (2.5th - 97.5th cent.)

- Thyroid **regulatory physiology is very sensitive** ie. serum TSH can be outside its reference range while FT4 & FT3 are still well within their's. Hence terms such as **sub-clinical hyper- & hypothyroidism**.
- The use of TSH **presumes that pituitary physiology is intact**.
- TSH can mislead in **hypopituitarism** & **thyrotrope atrophy** from endogenous or exogenous thyroid hormone XS.
- Measure **both TSH & FT4** in:

- hypopituitarism/hypothalamic disease
- thyrotrope atrophy due to chronically high thyroid hormone levels.
- pregnancy
- thyroid hormone resistance

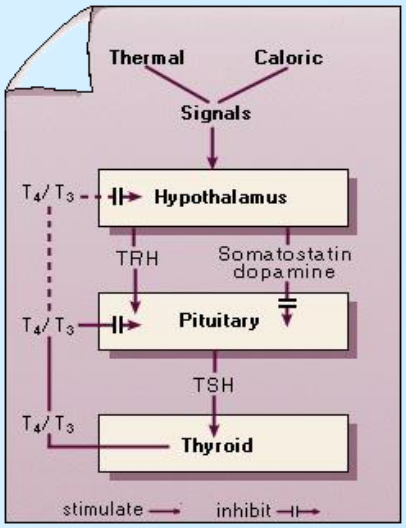
See *"The UK Guidelines for the Use of Thyroid Function Tests"*, available on the www, for evidence-based info. on the use & interpretation of TFTs in a wide range of conditions.

Causes of ↑

- primary hypothyroidism
- impairment of T4 to T3 conversion eg. due to amiodarone
- sick-euthyroidism especially at the start
- drugs
- interfering antibodies in the patient sample
- thyroid hormone resistance
- ectopic TSH secretion by tumours (very rare) eg. lung & breast

Causes of ↓

- exogenous thyroid hormones
- sick-euthyroidism
- primary hyperthyroidism
- drugs
- secondary hypothyroidism (pituitary hypofunction)
- tertiary hypothyroidism (hypothalamic hypofunction)



TSH receptor Ab 3d 1.5mL
See FT3, FT4 & TSH.

Sent

0.0 – 0.4 U/L
Sheffield Protein Reference Unit

Use

- Antibodies to TSH receptors can stimulate them & are associated with **Grave's disease**.

U

UIBC (unsaturated iron binding capacity) 3d 1.5mL Daily

See ferritin, folate, iron, iron satn, TIBC & vit B12.

Female	24.2 – 70.1 umol/L
Male	22.3 – 61.7
Roche Cobas UIBC method sheet V5	

Use

- Ix of **anaemia**, **iron deficiency** & **iron overload**.
- UIBC is not especially useful itself, but it enables estimation of transferrin saturation (iron satn.).

Background

- UIBC is the difference between the serum iron conc. & what it is when the transferrin in the sample is saturated
- Transferrin is commonly approx. 30% saturated.
- The new assay at SASH measures UIBC. The previous one measured TIBC.
- TIBC is easily calculated (UIBC + serum iron), but iron satn. is **a better marker** of iron stores than either UIBC, TIBC or iron alone.
- **Iron saturation** = $\frac{\text{serum iron}}{\text{serum iron + UIBC}} \times 100\ \%$

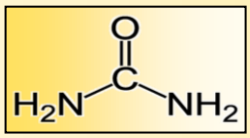
Interpretation

- See iron satn. & TIBC, & interpret those results in your patient.

urea (serum) 3d See profiles

See ALT, amino acids, ammonia, AST, comments, creatinine, eGFR, K, **MRI**, Na, PSA & uric acid.

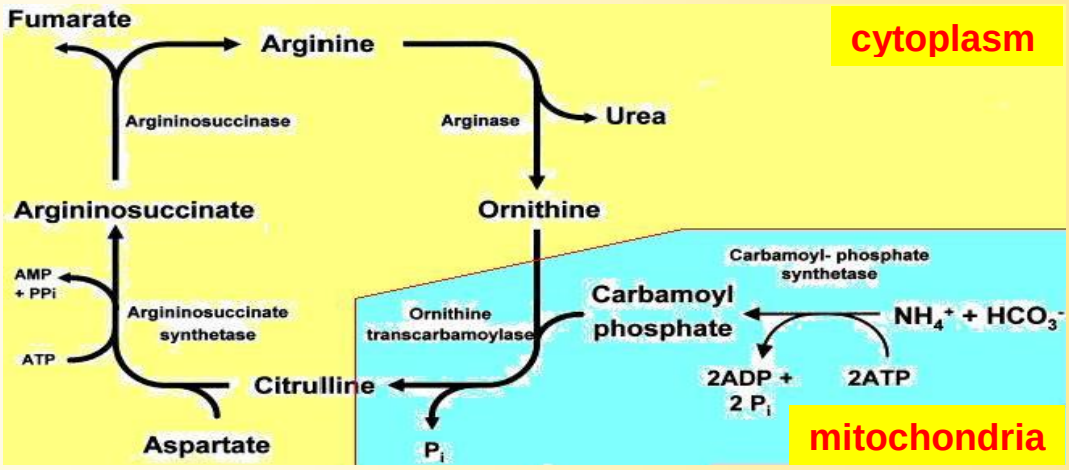
Daily



Children	Premature	< 2.7 mmol/L
	Neonates	< 7.0
	≤ 6 m	< 7.0
	> 6 m	< 8.0
Adults	≤ 65 y	< 8.3
	> 65 y	< 11.9
Roche Cobas UREAL method sheet V7 & Ref. Ranges for Adults & Children 2004		

Background

- The **deamination** (metabolism) of dietary & tissue amino acids produces **ammonia** which is **toxic**.
- **Conversion to urea** in the liver ↓ **its toxicity** & facilitates renal elimination.
- Ruminants secrete 25% of their urea into the gut where bacteria employ the ammonia released by urea-splitting microbes, for the synthesis of amino acids which are subsequently absorbed by the animal.
- Humans can only absorb the **ammonia produced by gut bacteria**, which contributes to the **load to be cleared**.
- A protein meal, g.i. bleed or tissue breakdown is followed by ↑ deamination, urea synthesis & ↑ serum urea.
- Urea is **synthesised** by a series of reactions termed the **urea cycle**, which progressively assembles 1 urea molecule with each "turn".
- In a urea molecule, 1 amino group comes from the amino acid **glutamic acid** (involved in carbamoyl phosphate synthesis) & the other comes from **aspartic acid** which enters the urea cycle at a later point. The amino transferases ALT & AST have key roles in the movement of amino groups to these 2 final steps into the cycle.



Ammonia (NH3) or ammonium (NH4+) ?

- Ammonia is **highly basic** (a strong proton acceptor).
- Thus under the *relatively* acidic body pH of 7.40, ammonia is **actually ammonium, NH_4^+**.
- ie. the familiar story of **urinary buffering by ammonia** secreted by renal tubules, **is untrue**.
- But not completely: excretion of nitrogen as NH_4^+ avoids synthesis of urea & production of hydrogen ions with it:

2 NH_3 + CO_2 + 3 ATP + H_2O → **urea** + 2 ADP + 4 P_i + AMP + **2 H** Minimisation of this is significant in acidosis.

Causes of ↑

- ↓ GFR (many factors influence levels in addition to GFR - impairs its use as a measure of renal function)
- GI bleed
- ↑ protein intake
- catabolic states eg. MI, burns, but ↓ long-term

Causes of ↓

- ↓ protein intake (esp. if carbohydrate high)
- pregnancy
- infancy
- acromegaly
- TPN
- severe liver disease
- malabsorption.

urea (24h urine) 3d 24h urine
See creatinine clearance & eGFR.

Daily

170 – 580 mmol/24h
Roche Cobas UREAL method sheet V7

This is **little used** for nitrogen balance studies these days because methodological & intra- & inter-individual variances diminish the value to the individual patient in practice.

urea (urine conc.) 3d 20mL
See creatinine clearance,
creatinine (U conc.), eGFR & urea (serum).

Daily

Morning urine	141 - 494 mmol/L
Roche Cobas UREAL method sheet V7	

Use

- Answering the question **"is there urine in this fluid?"** eg. surgical drain.
- Creatinine concentration can do the same thing.

Background

- Not a very useful test for other purposes (osmolality a better marker of water conservation & concentrating ability)
- Urine urea conc. is normally > **20x** the serum or tissue fluid conc.
- Small urine leaks may not ↑ the urea conc. in drain fluid as much as this ie. clinical judgement is important.

uric acid 3d 1.5mL
See ammonia, comments, oxalate, stones, thioguanine nucleotides, urea & UA urine.

Daily

Women	143 - 339 umol/L
Men	202 - 416
Roche Cobas UA2 method sheet V4	

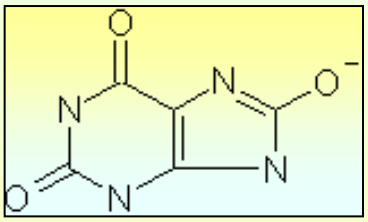
Use Ix of gout, tumour lysis, PET, tubulopathy, IEM

Background

- Humans, like many animals, **cannot degrade** the two heterocyclic rings of **purines** to carbon & nitrogen, so the molecules are recycled or excreted largely intact.
- Uric acid (**UA**) is the **final product of purine** (adenine & guanine) **metabolism in humans**. Other animals go a step further eg. reptiles & birds oxidise uric acid by means of *urate oxidase* to form **allantoin** which is more soluble & means that less water is needed for excretion. See DIAGRAM
- In contrast, **pyrimidines** (thymine, cytosine & uracil), which come from dietary & endogenous nucleic acids (DNA & RNA) & mononucleotides (ATP, GTP) have a single ring of alternating nitrogen & carbon atoms, which **can be metabolised** eventually to **urea** & **CO_2** or all or part recycled.

Gout

- UA has **low solubility** & is liable to form **crystals**, especially at sites of tissue injury eg. joints.
- **Serum levels do not correlate well with disease** ie. gout can occur at levels in the ref. range & symptom-free people can have clearly high ones. Additional **factors** regulate crystal formation & the response to it.
- In **primary hyperuricaemia** (it does not automatically cause gout), cases arise from 1) ↑ **UA production** due to over-activity of the purine-synthesising enzyme ***amidophosphoribosyl transferase,*** 2) ↓ **renal tubular excretion** of UA, 3) both & 4) **cause unclear** – most cases
- The **gender difference** is interesting: men have lower tubular secretion rates of UA, higher serum levels & higher prevalence of gout than women.



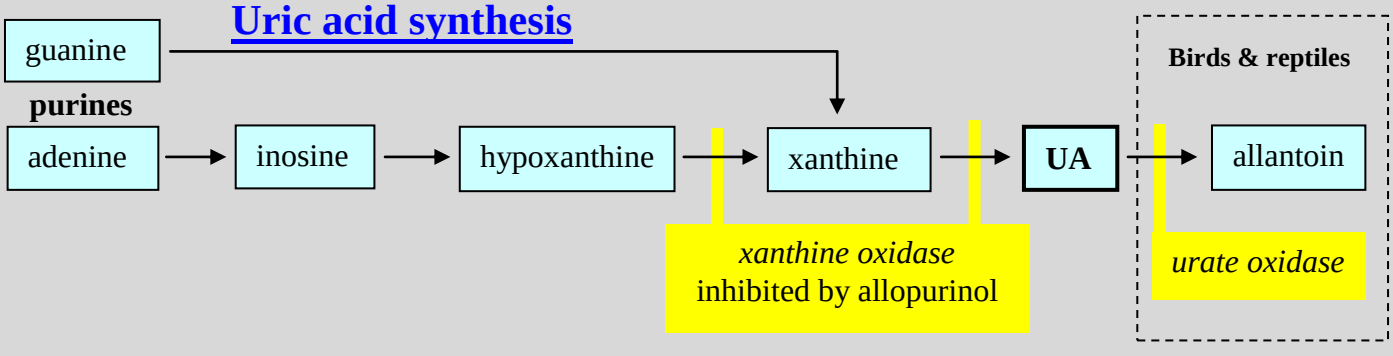
Causes of ↑

- Gout.
- Toxaemia of pregnancy.
- ↓ GFR.
- ↓ renal tubular excretion eg. thiazides.
- ↑ purine intake eg. high meat diet.
- ↑ DNA turnover eg. leukaemia, myeloma, lymphoma, polycythaemia & psoriasis.
- ↑ nucleic acid breakdown eg. tumour lysis, starvation, trauma.
- Inborn errors of metabolism eg. Lesch-Nyan syndrome (v. rare, deficient HGPRT, a purine recycling enzyme).

Causes of ↓

- Allopurinol (inhibits xanthine oxidase which ↓ UA synthesis).
- ↓ purine intake.
- Proximal tubular hypofunction (Fanconi syndrome, 80% of filtrate UA is normally reabsorbed in PCT).
- Hereditary renal hypouricaemia (defective tubular reabsorption/hypersecretion).
- Some malignancies eg. myeloma, Hodgkin's disease, bronchial carcinoma (may ↑ too, see above).
- *Xanthine oxidase* deficiency (catalyses UA synthesis from xanthine, v. rare).

Uric acid synthesis



uric acid (urine) **3d** **24h urine**

Daily

24h urine	1.2 – 5.9 mmol/24h
1st morning urine conc.	2.2 – 5.5 mmol/L
Roche Cobas UA2 method sheet V4	

See Ca (24h U), oxalate, PO_4 (24h U), stones & UA.

- **24h collection is best**
- **Intake dependent**

Children only

Children: **Age**	**UA/creat. ratio** (mmol/mmol)
1m – 6m	0.80 – 1.60
6m – 1y	0.70 – 1.50
1 – 2y	0.50 – 1.40
2 – 3y	0.47 – 1.30
3 – 5y	0.40 – 1.10
5 – 7y	0.30 – 0.80
7 – 10y	0.26 – 0.56
10 – 14y	0.20 – 0.44
14 – 17y	0.20 – 0.40
Matos V 1999 (5th – 95th centiles)	

- **24h procedure:**
  Urinate in toilet & note the time (can be any time). Collect all urine for the next 24h. The last is whatever can be passed at the noted-time next day.

Use

- Ix hyperuricaemia & paediatric & recurrent nephrolithiasis**.**
- Ix of acute UA nephropathy vs. other causes of ARF.

Background

- See uric acid (serum). DIAGRAM of synthetic pathway.
- ↑ UA in renal tubular fluid can precipitate & → nephropathy & ARF

Causes of ↑

- ↑ purine intake eg. meat, pate, roe
- ↑ UA production in tumour lysis eg. leukaemia, lymphoma, polycythaemia. Risk of ARF.
- ↑ UA production due to IEM eg. Lesch-Nyhan syn.
- ↓ reabsorption eg. Fanconi syn.
- Hereditary renal hypouricaemia (↓ urate-anion transporter function causes ↓ reabsorption in tubule).

urobilinogen 0d 5mL fresh urine
See bilirubin & porphobilinogen.

Daily

Negative

Test

- The lab. uses the same test strips as on wards & in GP surgeries ie. you can DIY.

Use

- Limited, but may help to **distinguish cholestasis from hepatitis** before LFT results are back,

Background

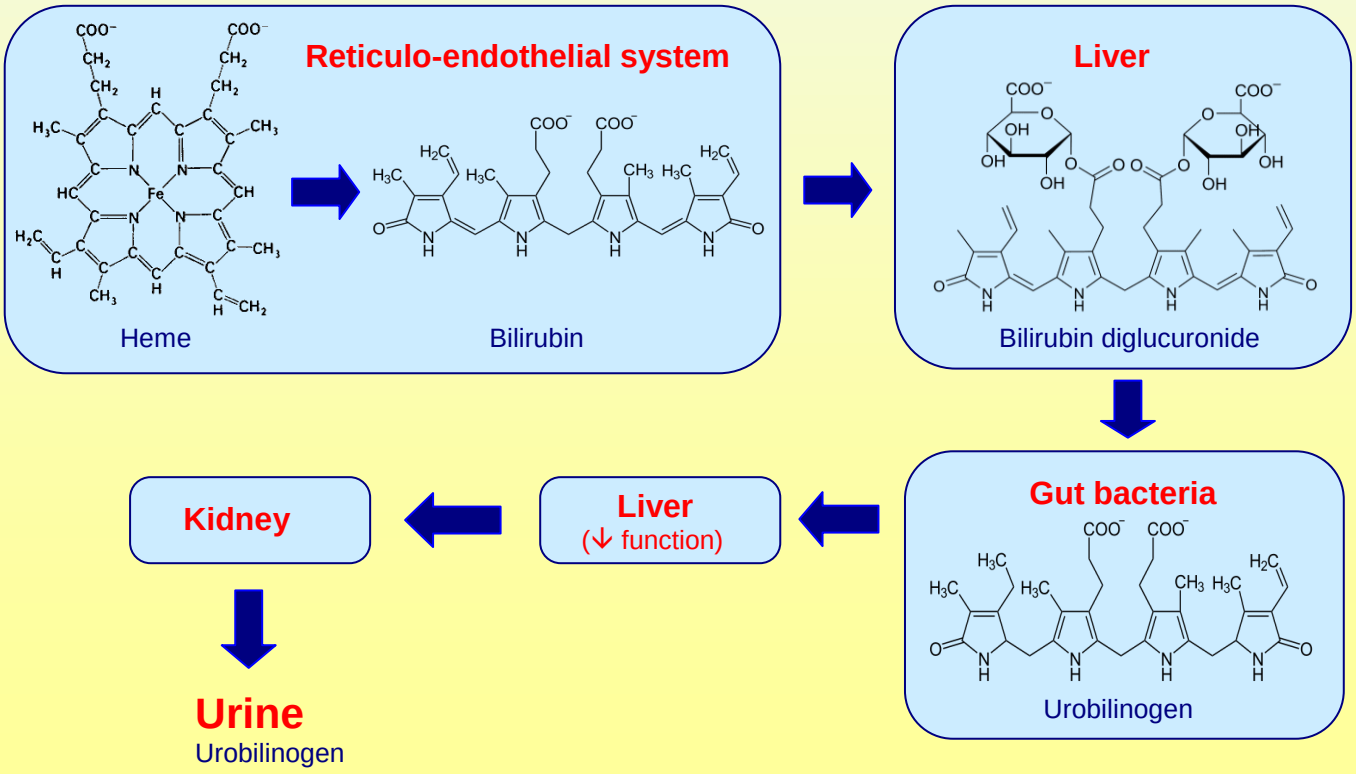
- **Haem** from Hb, myoglobin, cytochromes, catalase etc. is **degraded to bilirubin** (particularly in the reticulo-endothelial system) which is then taken up by the **liver** for **conjugation with glucuronic acid** & **biliary excretion**.
- **Bacteria in the gut** deconjugate & reduce the bilirubin to **urobilinogen**.
- **Reabsorbed** urobilinogen is normally swiftly cleared from portal blood by the liver before **re-excretion in bile**.
- In **hepatic dysfunction** this may be impaired such that urobilinogen reaches the systemic circulation where, being water soluble, it readily **enters urine**.

Causes of ↑

- **Hepatitis**, notably at an early stage before excretion of bilirubin & formation of urobilinogen has fallen.
- Major ↑ **in bilirubin production** eg. haemolysis *in vivo*, haematoma
- Hepatic **cirrhosis.**

Causes of ↓

- **Cholestasis**



V

vacuolated lymphocytes 0d 2mL

Sent

Refs & comment sent with result
Great Ormond Street Hospital

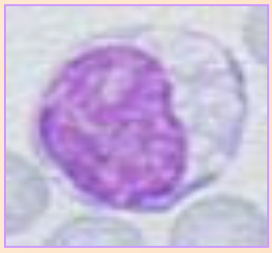
See GAGs, glycolipid, I-cell disease VLCFA & wbc enzymes.

Use

- **Screening** for **lysosomal storage disorders** (white cell enzyme defects).

Background

- Characteristically in these conditions, abnormally large quantities of **undegraded material** accumulates in the lysosomes & cytoplasm of lymphocytes & other cells.
- This is visible as large &/or ↑ number of cytoplasmic vacuoles in lymphocytes in peripheral blood films, on light & electron **microscopy**.
- **Examples of diseases** associated with ↑ vacuolation in descending frequency:



- Batten dis. (palmitoyl protein thioesterase)
- glycogenosis type II (Pompe disease, acid maltase deficiency)
- GM1 gangliosidosis (β galactosidase)
- galactosialidosis (α neuraminidase or β galactosidase)
- I cell disease
- glycoproteinoses

valproate 3d 1.5mL

Daily

50 – 100 mg/L
Roche Cobas VALP2 method sheet V9

See carbamazepine, lamotrigine, phenytoin & TDM.

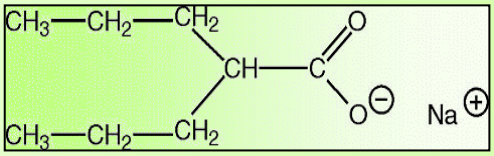
- **Pre-dose sample.**

Use

- Ix toxicity, non-compliance & loss of clinical effect or difficulty in establishing it (especially if on co-Px).
- **Not for routine monitoring**.

Background

- This **anticonvulsant & mood stabilising drug** acts at multiple sites in the brain eg. GABA metabolism, Na channels & DNA regulation.
- Valproate levels **correlate poorly** with clinical effects
- ie. **routine monitoring is not recommended**.
- **Toxicity** is increasingly likely above 100mg/L, but not certain.
- The main **side-effects** which impact the lab. are:



- hepatic dysfunction
- thrombocytopaenia.

- Valproate (& lamotrigine) inhibit *microsomal epoxide hydrolase* (mEH) causing:
- ↑ *carbamazepine epoxide* levels &
- ↑ clinical effect of co-prescribed carbamazepine.

Causes of ↓

- ↑ hepatic clearance due to enzyme induction by carbamazepine, phenytoin & phenobarbitone.

Causes of ↑

- mefloquine.

vancomycin 2d 1.5mL

Daily

mg/L
Roche Cobas c502 method sheet V10

See amikacin, comments, gentamicin, tobramycin & therapeutic drug monitoring.

Contact Microbiology for advice on interpretation & patient management.
See IPCAS on the SASH intranet for target levels, responses to results, doses etc.

Background

- A **glycopeptide antibiotic** for treating MRSA & Gram + ve infections in patients hypersensitive to penicillin.
- Serum levels aid Px of **effective doses with minimal side effects** eg. nephro- & ototoxicity, especially in
- the elderly
- patients with ↓ GFR (elimination is **80% renal**)
- children
- pregnancy
- when on additional nephrotoxic drugs.

- The **plasma half-life** in normal adults (**4 – 11h**) is ↑ by ↓ GFR eg. to as long as 10d in renal failure.

vasoactive intestinal polypeptide (VIP) 0d 6mL + aprotinin. Contact lab.
See gut hormone profile.
• **Keep samples on ice.**

Sent

< 30 pmol/L
Charing Cross Hospital

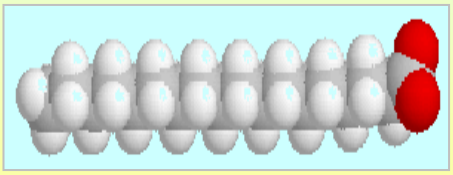
very long chain fatty acids 3d 1.5mL
See amino acids, GAGs, glycolipid, organic acids, vacuolated lymphocytes & wbc enz.

Sent

Chain length	**Conc.** (umol/L)
C22	30.5 - 97.7
C24	24.4 - 65.9
C26	0.15 - 0.91
C24/22 ratio	0 - 0.96
C26/22 ratio	0 - 0.022
phytanate	0 - 15.00
pristinate	0 - 2.00
Great Ormond Street Hospital	

Use
• For Ix **peroxisomal disorders** eg. adrenoleukodystrophy

Background
• Peroxisomes are **cytoplasmic organelles** containing **oxidative enzymes** for **breakdown** & **recycling** eg. of **fatty acids** by oxidation.
- Fatty acid carbon chains are shortened **2 carbons at a time**, with formation of acetylCoA
- ie. the chain lengths of VLCFAs accumulating as a result of defects of this process, differ by **multiples of 2**.
• Peroxisomes **also synthesize:**
- **cholesterol** & **bile salts** (in addition to the ER) &
- **plasmalogen**, a key phospholipid component of **myelin**, which may be why peroxisomal lesions are associated with myelin abnormalities.

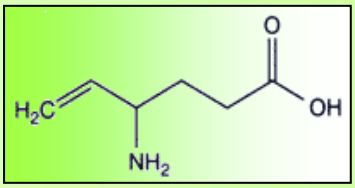


• Peroxisomal defects present in **neonates & adults**.
• The **clinical phenomena, severity & age** of onset depend on:
- **location** of the defective enzyme in the metabolic pathway
- **completeness** of loss of function
- **nature** of the lost function eg. cofactor binding, regulation, active site structure
• Thus, lesions at **different sites in the same enzyme** can cause **adrenoleucodystrophy** (ALD) which presents in children or **adrenomyeloneuropathy** (AMN) in young adults.

vigabatrin 3d 1.5mL
See amino acids (urine) & therapeutic drug monitoring.
• **Pre-dose sample.**

Sent

5 – 35 mg/L
St. Thomas' Hospital



Use
• **Routine monitoring is unecessary**. Use the dose as a guide.
• Assay may help in Ix of **non-compliance, treament failure** & **toxicity**.

Background
• An anticonvulsant.
• It **irreversibly inhibits** the enzyme *gamma-aminobutyric acid transaminase* which normally is responsible for the **catabolism of** the inhibitory neurotransmitter **GABA**.
• Vigabatrin **levels correlate poorly with clinical effect**, because of the irreversibility of its action.
• Plasma **halflife** is **6 – 8h**, but clinical effects change more slowly eg. 2 – 10d, because of this irreversible effect.
• 70% is excreted unchanged in **urine**.
• Serum **phenytoin** levels ↓ *ca.* 20% if vigabatrin is co-prescribed.

vitamin A (retinol) 3d 1.5mL

Sent

See bilirubin, carotene, GAGs, vit. D & vit. E.

- **Protect from light** with aluminium foil.
- **Stop supplements 2 d** before sampling.

< 7y	0.70 – 1.50 umol/L
7 – 12y	0.90 – 1.70
13 – 19y	0.90 – 2.50
Adult ♀	0.99 – 3.35
.. ♂	0.77 – 3.95
City Hospital Birmingham	

Use.

- Ix of vit A deficiency.
- **NOT an ideal guide to body stores**, because of variation related to the multiple sites of **retinol binding protein** synthesis eg. kidney, adipose tissue & muscle.
- **Vitamin D** level is as good a marker of fat soluble vitamin malabsorption as is vit. A.

Background

- Vit. A (**retinol**) is present in the diet eg. in **dairy** products, **eggs** & especially **liver**.
- Green & yellow parts of **plants** eg. carrots, are important sources of **carotenes**, which can be hydrolysed in gut mucosa to form vit. A.
- Retinol is **stored in the liver** & circulates bound to **retinol binding protein**.

Clinical effects of deficiency

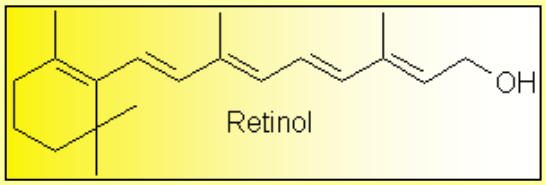
- Retinol in concert with the protein **opsin,** forms the visual pigment **rhodopsin**. This is **destroyed by light** & has to be replaced. Thus, retinol deficiency results in reduced vision in dim light.
- Vit. A is also needed for:

- **cell division**
- **cell differentiation**,
- **mucopolysaccharide synthesis & mucus secretion**.

Loss of the last functions causes dryness & hyperkeratosis of skin & most importantly, corneal drying (**xerosis**).
In severe deficiency, this is followed by ulceration & perforation (**keratomalacia**).

Causes of deficiency

- Inadequate diet
- Malabsorption eg. intestinal bypass, sprue
- Marked liver disease
- Gross proteinuria
- Deficient TPN.
- Preterm & small for dates neonates have low stores



vitamin B1 0d 2mL

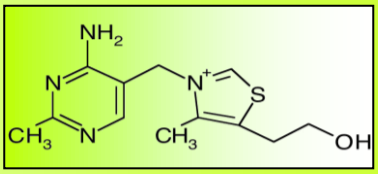
Sent

(thiamine). See vit B6 & vit B12.

- **Fasting needed.**
- **Protect from light** with aluminium foil.
- Take blood at ESH to **minimise delay** getting to the lab.

Adult	50 – 220 nmol/L
Deficiency: marginal	40
.. overt	≤ 5
Rotherham Hospital	

Use • Ix deficiency.



Background See thiamine for full details.

- Little stored in body.
- Sources: **cereals** & **meat**
- People at risk of deficiency: **alcoholics** (poor diet & inhibition of absorption) & renal **dialysis patients**
- Present as **free thiamine** in plasma & the bioactive form, **thiamine pyrophosphate** (TPP), in cytoplasm.
- TPP is a cofactor for enzymes involved in **carbohydrate metabolism** & **nerve function**.
- **Marginal deficiency** causes: confusion, irritability, malaise.
  Severe .. causes: beri beri, peripheral neuropathy, Wernicke's encephalopathy, Korsakoff's syn.

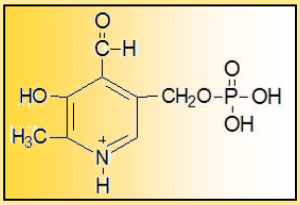
vitamin B6 0d 3mL

Sent

See ALT, AST, folate, oxalate, haem synthesis, thiamine (vit. B1) & vit. B12.

- **Protect from light** with aluminium foil
- Blood must be taken at ESH.

Adult	40 – 100 nmol/L
Hi risk of deficiency	< 20
Rotherham Hospital	



Use

- Ix **neuropathy** & **dermopathy**.

Background

- Present in food in **multiple forms**: pyridoxal, pyridoxine & pyridoxamine.
- Widespread in food. **Dietary deficiency is rare +++**
- **Isoniazid** & **penicillamine** are antagonists ie. prophylactic supplements needed.
- Converted to **pyridoxal 5-phosphate** (the bioactive form) in the body.
- Cofactor for many enzymes eg. AST, ALT, AGT, *δ amino laevulenic acid synthase* ie. involved in aminotransfer.
- Notable role in **serotonin** (5HT) & **tryptohan** synthesis.
- RDA = 15 ug/g of dietary protein. XS intake may cause neuropathy & rebound deficiency on stopping abruptly.
- Effects of deficiency: ↑ homocystine, ↓ Fe absorption. Clinical: Irritability, depression, skin scaling & ↑ pigment
- .. .. gross excess intake: peripheral neuropathy & encephalopathy.

vitamin B12 2d See profiles

See Co, elastase, ferritin, folate, homocystine, iron, iron satn, methylmalonic acid, **MRI**, thiamine, TIBC, UIBC, vit. B6 & Zn.

- **Ideally fasting**

Daily

191 – 663 ng/L
Roche Cobas Vitamin B12 method sheet V11 (2.5^{th} - 97.5^{th} cent)

Use

- Ix **anaemia**, **neuropathy**, **diet**, **malabsorption** & **replacement**.

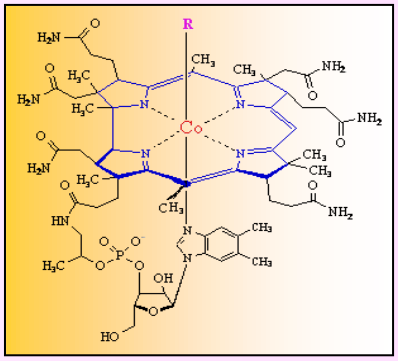
Background

- Vit. B12 is confined to the animal kingdom.
- **Liver, meat, eggs & milk** are good sources.
- **Vegans** can get sufficient B12 naturally from soil bacteria on their food.
- **Intrinsic factor**, from gastric parietal cells, protects dietary vit. B12 en route to absorption in the **terminal ileum**.
- Most vit. B12 in serum is bound to **transcobalamin I** & a little to **transcobalamin II**, which have roles in storage & transport to dividing tissues, respectively.

Clinical impact of deficiency

- Vit. B12 (actually **a mixture of cobalamins** eg. methyl, cyano, hydroxy, deoxyadenosyl) & folate are considered together, because both are B vitamins & essential for **erythrocyte maturation**. Deficiency of either causes **megaloblastic anaemia**, but vit. B12 deficiency also causes neuropathy. Indeed, folate administration may improve anaemia of vit. B12 deficiency but precipitate **subacute combined degeneration of the spinal cord**. Vit B12 & folate are cofactors in **RNA** & **DNA synthesis**, & **metabolism of amino acids** eg. methionine & cysteine.

Causes of ↓



- **Intrinsic factor deficiency** (pernicious anaemia) from: autoimmune disease, gastrectomy & atrophic gastritis.
- **Malabsorption:**
  - regional ileitis (Crohn's dis.)
  - gut resection
  - coeliac disease
  - small gut bacterial overgrowth
  - blind loop
  - pancreatic insufficiency
  - achlorhydria.
- **Pregnancy:** vit. B12 levels fall progressively by as much as 40% by the 3rd trimester ie. sometimes to well below the non-pregnant lower ref. limit, without apparent harm.
- **Dietary deficiency:** uncommon, but can occur in elderly people, vegans & those on extreme diets.

The "grey-zone"

- Vit. B12 levels in health & disease **overlap** (the grey-zone).
- Patients with evidence of vit. B12 deficiency eg. megaloblastic anaemia, can have levels in the lower end of the reference range & healthy people can have levels clearly below it.
- Variation in **transcobalamin II levels** may account for some of this.
- Vit. B12 **deficiency can take >1y to show clinically** ie. it may be too early for apparently healthy people with low vit. B12 levels to have become ill.
- **Clarification** may require Ix of causes & effects of deficiency eg.
  - **serology** for auto-Abs to intrinsic factor
  - a **therapeutic trial** of vit. B12
  - assay of **homocystine** & **methylmalonic acid** which show ↑ levels in functional deficiency, especially MMA.

Causes of ↑

- CKD
- hepatitis
- carcinoma
- severe CCF
- leukaemia
- cirrhosis
- B12 therapy
- polycythaemia vera
- liver metastases
- acute intake from diet (ie. should fast ideally)

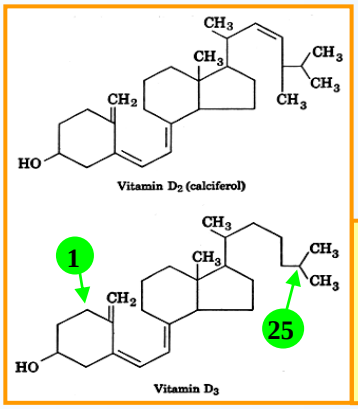
vitamin D_3 (25-OH vit D_3) 3d 1.0 mL

Twice a week

gold

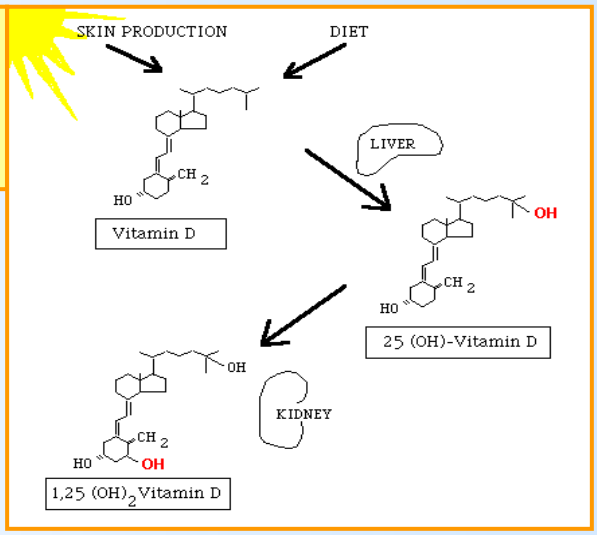
See ACE, Al, ALP, calcitonin, Ca, Ca adj, comments, **MRI**, PO_4, PTH, vit. A, 1,25-dihydroxy vit. D, vit. E & Zn.

- Assays **vit. D_2** ***plus*** **vit. D_3**



• severe deficiency	< 25 nmol/L
• insufficient, 2ndy hyperparathyroidism likely	25 – 50
• replete, but 2ndy hyperparathyroidism possible	50 – 75
• **optimum**	75 – 200
• possible toxicity if sustained	> 250
Roche Cobas Vitamin D total method sheet V5 & Vieth R et al 2007	

Vitamins D_2 & D_3 are the same at the all-important carbons 1 & 25, but differ a little internal to C25.



Use • A marker of **vit. D stores**.

Background

- There are **2 forms** of vit. D of importance.
- **D_2** (**ergocalciferol**) from **plants**. It is used to make some supplements.
- **D_3** (**cholecalciferol**) is synthesised in **skin** by the action of UV light on 7-dehydrocholesterol.
- Their differences do not affect biological function.
- Both are transported in plasma bound to **vitamin-D binding protein** & both require **hydroxylation:**
  - 1st in the **liver** at carbon 25 to form **25-hydroxy- vit. D**,
  - 2nd in the **kidney** at carbon 1 to form bioactive **1,25 dihydroxy vit. D.**
- 25-OH vit. D as a marker of **body stores**, is superior to measurement of PTH, which is less sensitive ie. PTH may remain in the ref. range after vit. D has fallen.
- But, PTH is used to Ix ↓ **1-hydroxylation** in **CKD**, which causes **2ndy hyperparathyroidism**. Plasma 1,25-OH vit. D is difficult to measure & even in health, the level. is **1000x lower** than that of 25-OH vit D.
- **Coeliac disease** has been reported to be twice as prevalent as clinically suspected & can cause deficiency of vit. D ie. check **coeliac serology**.
- Vit. D is **fat soluble** & 25-OH vit D levels ↓ with **fat malabsorption**. Indeed, it can be used as a marker of malabsorption of vitamin A & vitamin E, which are also fat soluble, but more labile & difficult & costly to assay.
- The clearest **role of vit. D** is in promoting **Ca** & **PO_4 uptake** from the gut, but there are less understood roles in bone, kidney, liver, skin, cell differentiation & the immune system.

Biochemistry of ↓ & ↑ vit D.

Biochemistry	Vit D deficiency	Vit D excess
serum Ca	↓ or lowish	↑
serum PO_4	↓ (or relatively low for the GFR)	↑
25-OH vit. D	↓	↑
PTH	↑	↓
serum ALP	↑ (late & insensitive marker of ↑ osteoblast activity due to osteomalacia)	N

vitamin D (1,25-OH vit D) 0d 4mL **No gel**  Sent

See ALP, Ca, PO_4, PTH & 25-OH vit D.

40 – 150 pmol/L
St Helier Hospital

Use

- **Very limited** eg. Ix of heritable forms of rickets, sarcoid hypercalcaemia.

- Use serum **25-OH vit D** to Ix **vit D intake** & **stores**.
- Use plasma **PTH** to Ix ↓ **1-hydroxylation** in CRF.

Background

- 1,25 dihydroxy vitamin D is the **bioactive** form of vitamin D formed by hydroxylation first at carbon 25 in the liver & then at carbon 1 in the kidney. See DIAGRAM
- **Not for assessment of vitamin D nutritional status** (use 25-hydroxy vitamin D), but it may help to uncover rare downstream defects eg. **receptor insensitivity**.
- **CKD impairs 1-hydroxylation** of 25-hydroxy vit D. The **2ndy hyperparathyroidism** this causes, enables PTH to be a marker of ↓ 1-hydroxylation, instead of 1,25-OH vit D itself (**1000x** lower conc. than 25-OH vit D).

vitamin E (tocopherol) 3d 1.5mL  Sent

See vitamin A & vitamin D.

- **Protect from light** with aluminium foil.
- **Stop supplements** 2 d before sampling.

< 2y age	11.5 – 24.4 umol/L
2 – 6y	7.0 – 21.0
7 – 12y	10.0 – 21.0
13 – 19y	13.0 – 24.0
Adult	9.5 – 41.5
all ages	Vit. E/chol. ratio ≥ 2.22 umol/mmol
City Hospital Birmingham	

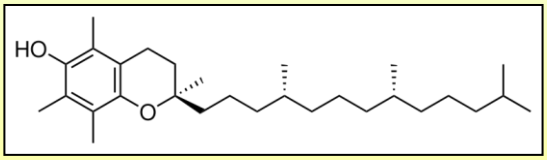
Use

- An **imprecise marker** of body stores. See below.

Background

- α-tocopherol is the most abundant form of an **8-member family** of compounds with vitamin E activity.
- **Widely available** in food. A human condition caused by *dietary* inadequacy is doubtful.
- Like the other fat soluble vitamins (A, D & K), it enters the body in **chylomicrons** & **beta-lipoproteins** with lipids from the gut, via **lymphatics**. Vit. E is transported with lipoproteins in the peripheral circulation, which means that its level ↑ & ↓ with cholesterol. This distortion is lessened by expression of vit. E as a **ratio to cholesterol.**
- Vit. E **stores are widespread** & can supply needs for **months**.
- Vit. E **protects lipids** from **oxidative damage**: evidence of deficiency in animals can be reversed by other antioxidants eg. selenium, ie. vit. E in lipoproteins does more than just hitch a ride.

Causes of deficiency



- **Fat malabsorption** eg.
- small intestinal disease
- short gut
- bariatric surgery
- pancreatic insufficiency
- biliary cirrhosis
- chronic cholestasis (the associated hyperlipidaemia can maintain serum vit. E levels despite low body stores).
- **Effects of deficiency** eg. areflexia • gait disturbance • ↓ proprioception • ↓ vibration sense • posterior column & peripheral nerve degeneration.

Causes of toxicity

- Supplement excess

VMA 3d acidified 24h urine
(shorthand for catecholamines)
See adrenaline, aldosterone, CgA, 5HIAA, noradrenaline & renin.

Children only, 20mL urine


Sent


See below.

noradrenaline	(norepinephrine)	<500 nmol/24h
adrenaline	(epinephrine)	<100
dopamine		<3000
normetadrenaline	(normetanephrine)	<3.3 umol/24h
metadrenaline	(metanephrine)	<1.2
3-methoxytyramine		<2.5
St Helier Hospital		

Use • Ix atypical **hypertension** (phaeochromocytoma).
• **neuroblastoma**

Urine collection

DIAGRAM of catecholamine metabolism

- 24h urine in a **plain** bottle.
- **Procedure**: Urinate in the toilet & note the time (can be any time). Collect all urine for the next 24h. The last one to collect is whatever can be passed at the noted-time next day.
- Protection needed only from direct sunlight.
- Keep cool, but fridge is unnecessary.
- At room temp. without acid, catecholamines are stable for the collection-duration & metabolites for several days.
- Take to lab. on the day of finishing.
- Acid preservative is **added in the lab**. when the sample is handed in.
- **2 collections** are sufficient for exclusion in most patients even if symptoms are episodic.
- **Urine collected after a clinical episode is especially valuable**. Start collecting asap, as above.
- In **children**, 20mL of fresh urine can be used to measure catecholamine/creatinine ratios because the difficulty in collecting truly 24h specimens negates their superiority. **Age related ref. info. is sent with the results**.

Interferences to avoid:

- **Vanillin** in icecream etc. **does not interfere** in modern assays.
- **Bananas**, **pineapple**, **citrus fruit, cocoa, chocolate** & **nuts** (rich in catecholamines) for 2d before & during the collection. Dietary catecholamines contribute little to urine levels because they are metabolised by the liver to **metadrenaline** & **normetadrenaline** on first-pass, but these metabolites may give **false + ves** in the initial screen.
- **Heavy exercise**, **smoking**, **tea**, **coffee** & **stress**, which stimulate physiological catecholamine secretion.
- **High protein meals** & **high vitamin C intake**, which ↑ dopamine excretion (**pregnancy** does this too).

Drugs – always give details.

- **β-blockers, ACEI, ARB** drugs & metabolites can cause analytical interference & apparent ↑ in metadrenaline
- **doxazosin** & **felodipine** - ↑ normetadrenaline
- **imipramine** & **prochlorperazine** - ↑ metadrenaline, normetadrenaline & methoxytyramine.
- **levodopa** & **methyldopa** - ↑ 3-methoxytyramine, HVA & dopamine
- **methylphenidate**, **amphetamines** & **derivatives** eg. **ephedrine** - ↑ normetadrenaline

Test • **Step 1.** Urine **metadrenalines** (metadrenaline, normetadrenaline & 3-methoxytyramine) are assayed (**sensitive** but not specific because ↑ dietary catecholamines will ↑ these metabolites too).
Step 2. If any metabolite is > 95th centile of the reference population, **free catecholamines** are measured too (**specific** ie. they reflect endogenous catecholamines, not dietary ones which are metabolised).
• **Free catecholamines** & **their metabolites** (metadrenaline, normetadrenaline & 3-methoxytyramine) have **superior** diagnostic performance to **VMA** (a catecholamine metabolite) which is little used now except to Ix children.

Background

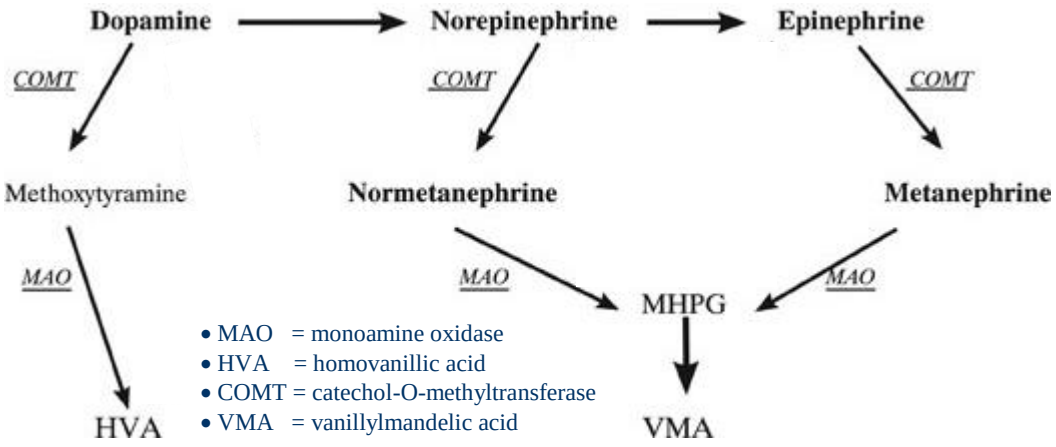
- **Phaeochromocytomas** are tumours of chromaffin tissue, which are **mostly non-malignant**. 90% arise in the **adrenal medulla**. In contrast, **neuroblastomas are highly malignant**, present in childhood, arise in the **sympathetic** nervous system & only 40% are adrenal.
- Hypersecretion of catecholamines (noradrenaline & adrenaline) by **phaeochromocytomas** cause a characteristic **syndrome**: paroxysmal (occasionally persistent) hypertension • anxiety • sweating • tremor • tachycardia • palpitations • throbbing headache • facial palor/flushing • hyperglycaemia. It is a cause of surgically curable hypertension in young adults.
- This syndrome is **uncommon** in **neuroblastoma,** even though plasma catecholamine levels may be just as high.
- **Neuroblastomas** secrete **dopamine**, particularly.

VMA continued

Sources of catecholamines & metabolites in urine

- **Dopamine** - substantial amounts are secreted into urine by the **normal** & **abnormal kidney**. Unlike most urinary catecholamines, dopamine from the kidney is **unconjugated** (sulphated) because it has not been to the **liver** where conjugation takes place. ↑ **dopamine** excretion is characteristic of **neuroblastoma**, but it can also occur in phaeochromocytoma.
- **Noradrenaline** *(norepinephrine)* comes from the adrenal medulla & incomplete reuptake by postganglionic sympathetic nerves.
- **Adrenaline** *(epinephrine)* is the main product of the chromaffin cells of the adrenal medulla.
- **Normetadrenaline** *(normetanephrine)* & **metadrenaline** *(metaneprine)* are noradrenaline & adrenaline metabolites, respectively, from liver & muscle.
- **3-methoxytyramine** is a **dopamine** metabolite which ↑ with ↑ dopamine secretion by non-renal disease ie. it helps in the **distinction of renal from non-renal** causes of ↑ urine dopamine excretion.

The biochemical relationship between catecholamines & their metabolites:



W white cell enzymes

white cell enzymes 0d 5-10mL

Sent

See amino acids, ammonia, GAGs, α-galactosidase A, GAL-1-PUT, I-cell disease, organic acids, vacuolated lymphocytes & VLCFA.

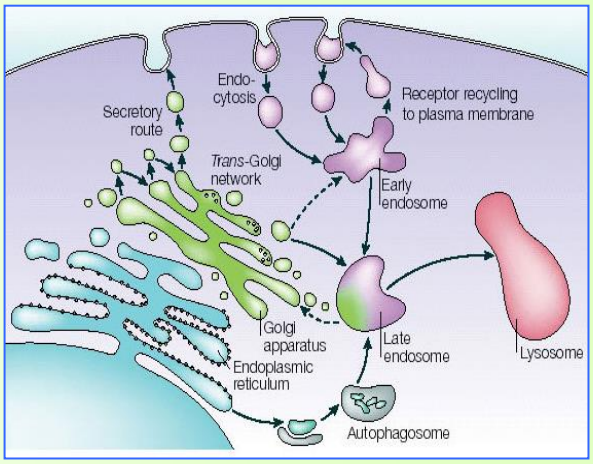
- **MUST** be in ESH lab. by **10am.**
- **MUST** get to GOSH by **2pm.**
- **DO NOT** send on **Friday or at w/e**
- **Full clinical details** help the reference lab. To give a full service.
- **Invalidated** by blood transfusion in the previous 6 w.

Refs & comment sent with result

Great Ormond Strret Hospital

Background

- Circulating w.b.c. give convenient access to lysosomes which are organelles with **multiple catabolic functions** involving eg. mucopolysaccharides, glyco-proteins & glycolipids.
- Deficiencies of the enzymes which catalyse these functions lead to accumulation of substrates & "**lysosomal storage diseases**" eg. Gaucher's, Fabry's & Hurler's syndromes.
- Enzyme deficiency can be multiple too eg. I cell disease.
- Whilst white cell function may not be much affected, tissues with more need of the impaired enzyme, suffer damage eg. neurons in Tay Sachs disease (*hexosaminidase A* deficiency).



- **Early in disease**, the clinical features may not be diagnostic, but at this stage a **biochemical diagnosis** can be most useful eg. for prognosis, to enable appropriate therapy to slow progression & for planning further children.
- **Enzyme replacement** is possible in some conditions eg. Pompe's disease (acid maltase deficiency).
- Lysosomes don't just grind macromolecules in conjunction with another intracellular compartment, **endosomes**, which contain endocytosed extra-cellular, intra-cellular & plasma membrane components eg. ligand-receptor pairs. They are also important for **recycling** macromolecules (saves energy), **signal transmission** (separation of ligand from receptor), **plasma membrane repair** & calcium-dependent **secretion** (rapid) in "non-secretory" tissues eg. lymphocytes. This is distinct from the familiar regulated pathway of secretory tissues (see chromogranin A).
- It is unsurprising that lysosomal storage diseases have such clinical variety & gravity, in view of these functions.

X

xanthochromia (CSF)

xanthochromia (CSF) 1d 0.5 mL CSF

Daily

See bilirubin, glucose (CSF) & CSF protein.

- **Protect from light** with Aluminium foil.
- **Mon – Fri.** Get to lab. by 4 pm
- **Sat. + Sun.** .. .. 12 noon.

NB. Only available if appropriately experienced staff are present.

Interpretation
Cruikshank et al 2008

Use • Ix of **SCAN-NEGATIVE SAH** (subarachnoid haemorrhage).

Test:

Do

- **Give CT scan result** with request. **MUST BE "SCAN-NEGATIVE".**
- **Protect from light** with aluminium foil (light degrades bilirubin).
- **Send the least blood stained** specimen of CSF (usually the last tube).
- **Note progressive clearing** of blood as CSF flows out ie. trauma.
- **Interpret results in the full clinical context & with due caution**. This test operates at the limit of detection, which impairs certainty.

Don't

- Don't collect CSF **within 12 h** of onset of symptoms of SAH (beware **false negatives**). Formation of **bilirubin** (the principal marker of SAH) from rbcs in CSF is **not instant**.
- Don't use the **vacuum tube system** (risk of haemolysis if heavily bloodstained).

Background

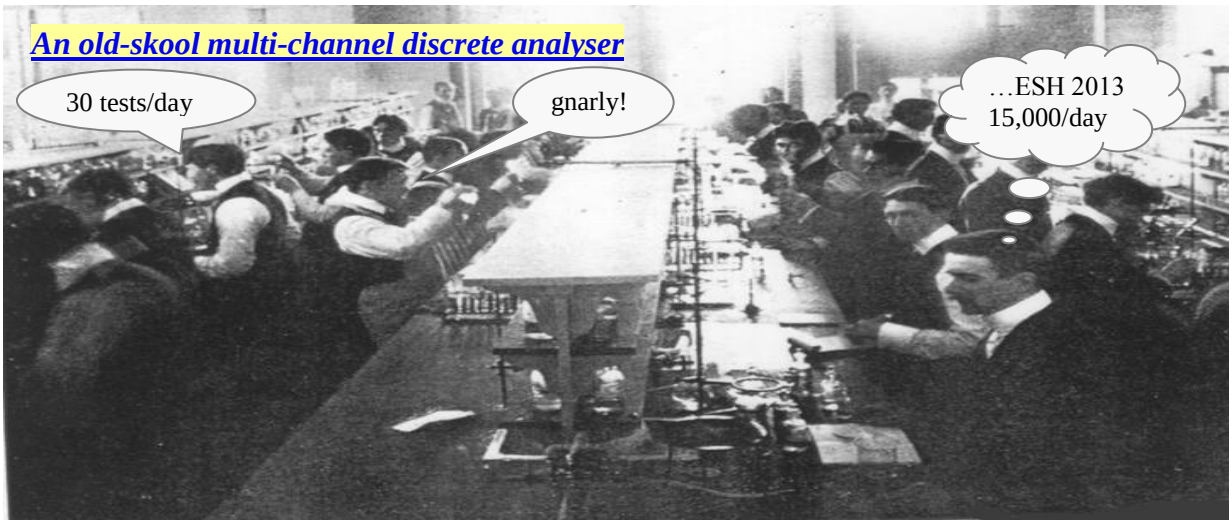
- **CT scanning is the first-line Ix of SAH** & examination of CSF for bilirubin **does not add to a + ve scan**.
- **5 %** of patients with SAH are **scan-negative** in the 24h after symptom onset.
- Xanthochromia is naked-eye visible **yellowing** due to bilirubin produced by degradation of haem in spilt-blood.
- In scan-neg. cases, **visible xanthochromia is unlikely** & **CSF looks clear** because the haemorrhage is small & the CSF changes slight. **Spectrophotometry** is needed to detect the low levels of bilirubin.
- **Only haem released *in vivo*** is metabolised to bilirubin. This is first seen in CSF 9 – 10h after SAH. Thus, bilirubin sheds light on the source of blood in CSF, which can come from L.P. trauma too.
- **CSF bilirubin is a more specific marker of SAH than Hb**, but results can ↑ in the absence of SAH, with:

- ↑ **serum bilirubin**
- ↑ **serum protein** &
- ↑ **CSF protein**

- **A big oxyHb peak** on spectrophotometry (whatever the source of blood) **obscures the bilirubin peak**, which is likely to be small in scan-negative patients ie. **minimally blood-stained CSF is crucial**.
- Despite these interferences, the results, when combined with **clinical judgement**, allow the majority of scan - ve patients to safely avoid having cerebral angiography.

Y **yesteryear**

An old-skool multi-channel discrete analyser



Z

zinc
zonisamide

zinc 3d 1.5mL
See ALP, CRP, folate, **MRI**, Se, vit B12 & vit. D.

Sent

11 – 24 umol/L
Royal Surrey County Hospital

Use • **Ix deficiency.**

Background

- **Key roles** in **enzyme** function, cell **membranes**, cell **signalling**, **nerve** function, **nucleic acid** synthesis & protein/ nucleic acid & protein/protein **interactions** eg. zinc-fingers in transcription factors.
- The activity of **> 200 enzymes** depends on zinc ions eg. ALP, *carbonic anhydrase*, *RNA* & *DNA polymerases*, *alcohol dehydrogenase*.
- A healthy adult has **2 – 3 g** of Zn in total, **> 80% in bone & skeletal muscle**.
- **RDA:** neonates 2mg/d, adults 10 - 15mg/d.
- **Sources:** well absorbed from red meat, poultry, nuts & legumes.

- **phytates** ↓ absorption by binding Zn ions.
- dietary **copper** & **iron compete** for transport mechanisms, causing ↓ Zn uptake.

Causes of deficiency

- **Absorption is normally weak** eg. it can be as little as 20 %, especially in **vegetarians**.
- Predisposing factors eg. alcoholism • renal failure • liver disease • cancer • corticosteroid Px.

- Even in the West, Zn deficiency readily occurs.

- **Inadequate diet**
- **Malabsorption** eg. coeliac disease, short bowel
- ↑ **loss** eg. Crohn's disease
- ↑ **need** eg. pregnancy, lactation, recovery from catabolic states.

Clinical evidence of deficiency:

- ↓ growth
- ↓ night vision
- ↓ wound healing
- ↓ sense of taste & smell
- Characteristic skin rash
- ↓ immune function.

Causes of ↓ plasma Zn in addition to ↓ stores:

- ↓ albumin conc. (binds 80% of plasma Zn)
- acute phase response (↑ tissue & liver uptake of Zn & ↓ albumin conc.)
- Menstruation
- Pregnancy – a **physiological** effect (↓ albumin), but **true deficiency** can occur in this state of ↑ need.

Causes of ↑ plasma Zn in addition to ↑ stores:

- Diurnal variation – peak at about 10 am.
- Tissue breakdown.
- Post-prandially.

zonisamide 3d 3mL **No gel**  Sent

15 – 40 mg/L
St. Thomas's Hospital

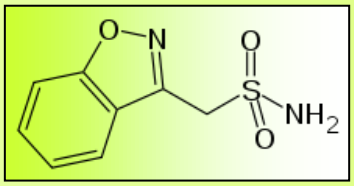
See carbamazepine, cyclosporin, lamotrigine, phenobarbitone, phenytoin & TDM.

- **Pre-dose sample.**

Use

- **Not for routine monitoring**.
- Ix toxicity, non-compliance & loss of clinical effect or difficulty in establishing it, especially with co-Px.

Background



- Sulphonamide related.
- Adjunctive therapy for **epilepsy.**
- Inhibits Na & Ca gated channels and also has carbonic anhydrase activity.
- Well absorbed.
- **30% excreted unchanged in urine**.
- Metabolised in the **liver** by **CYP3A4** & then **glucuronidated**.
- Plasma **halflife = 65h** in the absence of alteration by other drugs. See below.

Causes of ↓

- Induction of CYP3A4 by eg. phenytoin, carbamazepine & phenobarbitone can **halve halflife**.

Causes of ↑

- Inhibition of CYP3A4 by eg. lamotrigine, ketocanazole & cyclosporin.

Biochemical side-effects

- Renal calculi
- Metabolic acidosis

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PHOTOGRAPHS

Dr. Robert S. Jackson, Consultant Chemical Pathologist

Qualifications:

• BMed Sci	Sheff.	1979
• MB, ChB	Sheff.	1980
• FFARCS	Eng.	1984
• MSc Clinical Biochemistry	Lond.	1993
• MRCPath.	Lond.	1996
• FRCPath.	Lond.	2006

Special interests:
Neuroendocrinology of obesity, skin & hair.

Achievements:

- First cases of *PC1* mutation ahead of murine k.o.
- First description of full gDNA organisation of h7B2.
- First use of plucked-hair for quantitation of neuroendocrine mRNA expression in otherwise inaccessible tissue.
- The series of user-friendly encyclopaedic handbooks.
- A pathology service of leading efficiency.

Mr. Arnold Rady, Lead Biomedical Scientist, Chemical Pathology

Mr. Mike Rayment, Pathology Services Manager & Quality Manager

Mr. Eddie Onipede, Lead BMS for Haematology

Mr. Martin Stone, Pathology Computing Manager

Ms. Kerri Campbell-Bowler, Phlebotomy Manager

Reception for *all* Pathology samples & booking of Chemistry & Haematology requests.

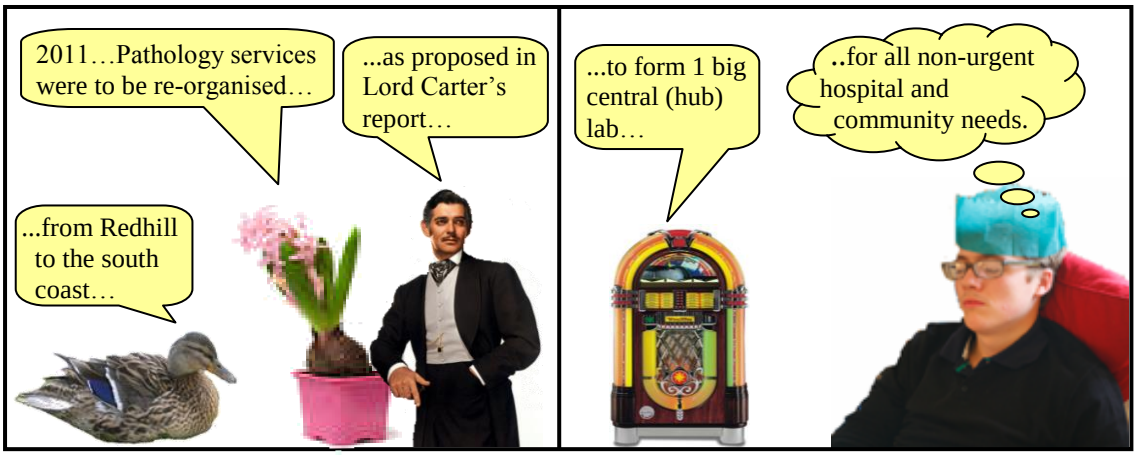
See service availability

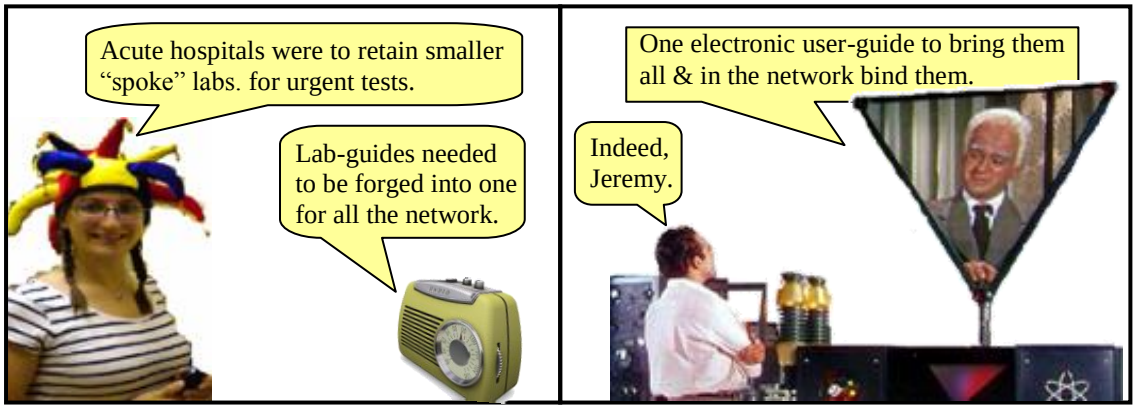
Staffed

- Mon. – Fri. 8am – 8pm
- Sat. 9am – 1pm
- Sun. 9am – 1pm

Sarah, Carol & Jacqui (L to R).

EPILOGUE





TOP

MINIMUM RE-TEST INTERVALS (MRI) & related info. Based on ACB 2013

- Evidence-based guidance & references on when & how often to request tests, with the aim of avoiding unconstructive repetition & omission of significant tests.
- Does paint dry quicker if it is checked more often? Physiological & pharmacokinetic processes take time too. Testing hastily before equilibration is unnecessary & can give misleading results.

BONE PROFILE & VIT. D See Liver. Bone profile = ALP, Ca, PO_4 & albumin.

TEST	MRI + ADVICE	REFS
Bone profile: non-acute setting without other specific clinical indications.	**3 months**.	ACB 2013
Bone profile: acute setting.	**2 d**.	ACB 2013
Bone profile: acute hypo/ hypercalcaemia, TPN & ITU patients.	May require more frequent monitoring.	ACB 2013
ALP & **total protein** in acute setting.	**1 w**. ALP may need checking more often eg. in the context of acute cholestasis.	ACB 2013
Vit. D: no clinical signs or symptoms.	**Do not retest** unless initial result is ↓ & a subclinical cause is found eg. coeliac.	
Vit. D: cholecalciferol or ergo-calciferol Px, where baseline serum vit. D was *adequate*.	**Do not retest**, unless clinically indicated e.g. sick, coeliac or Crohn's patient.	Sattar 2012 Sattar 2012a
Vit. D: cholecalciferol or ergo-calciferol Px where baseline serum vit. D is ↓ & there is disease that might ↓ absorption.	**3 – 6 months**.	ACB 2013
Vit. D: calcitriol or alphacalcidol Px.	**Do not measure** vit. D.	ACB 2013

CARDIAC

TEST	MRI + ADVICE	REFS
cTnT: acute coronary syn. (ACS)	• See cTnT for protocol. • High sensitivity (hs) cTnT assays usually require multiple samples with a 2nd test in 3 – 6 hr after the first at presentation.	Hamm 2011 Thygesen 2010
cTnT: Renal failure.	• cTnT can ↑ in CKD, especially with hs assays. See cTnT. • Serial samples may be needed to Ix ACS. See cTnT.	Khan 2005
NT-ProBNP: primary care heart failure (HF) triage.	• Test once unless there is another episode of suspected HF after the previous diagnosis of HF has been excluded. • Single time point use is adequate for NICE guidance purposes.	NICE CG108

DIABETES

TEST	MRI + ADVICE	REFS
Screening in asymptomatic patients: • Adults < 45y, normal Wt. (BMI<25 kg/m^2) no risk factors.* • Adults > 45y normal Wt., no risk factors.* • Adults >18y, BMI ≥25 kg/m^2, 1 risk factor.*	• **Not recommended**. • **3 y**. • **3 y** if result is normal.	ADA 2012 * Risk factors for DM in table 4 page S14 of this ref.
HbA1c: **diagnosis** in asymptomatic patients. **Not for children & young adults.**	**Do not base a diagnosis on only 1 abnormal HbA1c** (or plasma glucose) result**.** Confirm with at least 1 more result in the diabetic range within 2 w of the first, either fasting, random or an OGTT.	WHO 2011
HbA1c: monitoring type 2 DM.	**2 – 6 monthly**. A test done <3 months indicates direction of change rather than a steady state. 6 monthly intervals once the blood glucose conc. & therapy are stable.	NICE CG87
HbA1c: monitoring type 1 DM in children & young people.	**2 months**.	NICE CG15

eGFR

TEST	MRI & ADVICE	REFS
eGFR by MDRD formula. **Invalid in AKI**	Repeat in **14d** if ↓ eGFR is a new finding or previous result was < 60 mL/min/1.73m^2.	NICE CG73
eGFR: radiological procedures/contrast administration	See local guidelines & Royal College of Radiologists guidelines.	RCR 2010
eGFR: Cockcroft & Gault formula.	For estimating chemotherapy & drug dosage. Test valid up to 24h before dose unless creatinine level or fluid balance change rapidly.	BNF

GASTROINTESTINAL

TEST	MRI + ADVICE	REFS
Faecal **elastase**	**6 months**	Molinari 2004
Faecal **calprotectin**	**6 months**	van Rheenen 2010
Cu, **Zn**, **Se**	At **baseline** & **2–4 weekly** depending upon results.	NICE CG32
Ferritin: haemochromatosis monitoring.	EASL recommends initially **3 monthly** but more often as ferritin approaches normal range eg. monthly.	EASL 2010
Ferritin: iron deficiency diagnosis.	**Do not repeat** unless diagnosis is doubted.	Goddard 2011
Iron profile/ferritin: parenteral nutrition monitoring.	**3 – 6 months**.	NICE CG32
Iron profile/ferritin: CKD.	Monitor iron status no earlier than 1 w after iv. iron & then 1 – 3 monthly.	NICE CG114
Iron profile/ferritin: normal patient.	**1 y.**	NICE CG32 Smellie 2006
vit B12 & folate: monitoring deficiency Px.	**Do not retest** vit B12 &/or folate in deficiency Px unless compliance doubted or anaemia recurs.	CKS 2013
For more guidance on the lab. monitoring of patients on nutritional support, particularly parenteral nutrition & those receiving enteral or oral feeds who are metabolically unstable or at risk of re-feeding syndrome, please see NICE CG32.		

LIPIDS FLP = fasting cholesterol (total), HDL chol., LDL chol. & Tg.		
TEST	**MRI + ADVICE**	**REFS**
FLP: for low risk cases for CVD assessment.	**3 y**	www.bettertesting.org.uk
FLP: for high risk cases for CVD assessment & those stable on Px.	**1 y**	ACB 2013
FLP: at start or change of Px.	**1 – 3 months**	ACB 2013
Tg: to assess effect on hypertriglyceridaemia of diet & alcohol changes	**1 w**	ACB 2013
Tg: patients on TPN or who have hyper-triglyceridaemia pancreatitis.	**1 d**	ACB 2013

LIVER See bone profile. LFT = ALT, ALP, albumin + total bilirubin.		
TEST	**MRI + ADVICE**	**REFS**
LFT: non-acute setting.	**1 – 3 months**.	Smellie S 2011
LFT: acute inpatient setting.	**3 d**.	ACB 2013
GGT & **conjugated bilirubin** in acute setting.	**1 w**.	ACB 2013
LFT: acute poisoning, TPN, acute liver injury & ITU patients.	**1 d**.	ACB 2013
LFT: neonatal jaundice	Recommendations above are unsuitable.	

PREGNANCY		
TEST	**MRI + ADVICE**	**REFS**
β-hCG: **urine** pregnancy test.	**– ve**: repeat in 3d or at least 28d after start of LMP.	ACB 2013
β-hCG: **serum** …..ditto…...	**+ ve**: don't repeat. **– ve**: repeat in 3d if no period.	Serum hCG doubles in 1.5 – 2d.
β-hCG: serum ectopic pregnancy	48 h repeat interval. Serum hCG doubles in 1.5 – 2d.	RCOG 2004 Mol B 1999
LFTs in obstetric cholestasis.	Test wkly until delivery once obstetric cholestasis diagnosed. Postnatally, defer LFTs for at least 10d.	RCOG 2011
Persistent pruritus & normal biochem	LFT: test every 1–2w.	RCOG 2011
Bile salts: obstetric cholestasis.	x1/w monitoring. x2/wk later & if clinical state changing.	ACB 2013
Urate: in pre-eclampsia.	Awaiting expert advice whilst not admitted: twice weekly urate.	ACB 2013
Urine protein: in pre-eclampsia.	At each antenatal visit to screen for pre-eclampsia. Once diagnosed do not repeat quantification of proteinuria. However, test daily in severe ↑ BP.	NICE CG62 NICE CG107
LFT & **U+E**: in pre-eclampsia.	At least daily when results abnormal. More often if condition worsens. • mild hypertension, test twice weekly. • moderate hypertension, test x3 /w • severe hypertension test >x3 /w	NICE CG107
Hyperthyroidism: monitoring Px (UK)	**On anti-thyroid drugs.** Test TSH + FT4 prior to conception & asap when pregnant. Monthly repeat tests until stabilised. **Diagnosed in pregnancy**. Test TSH + FT4 (FT3 as indicated) monthly until stabilised.	ACB 2006
Hyperthyroidism: monitoring Px (USA)	**On anti-thyroid drugs**. Test TSH + FT4 (FT3 as indicated) approx. 2 – 6 wkly.	Stagnaro-Green 2011
Hypothyroidism: monitoring Px.	• Test TSH + FT4 to assess thyroid status & monitor T4 Px. • **MRI = 4w** • FT3 testing is inappropriate. Recommended TFT time points: • before conception • diagnosis of pregnancy • antenatal booking • at least once in 2nd & 3rd trimesters. • after delivery • new diagnosis: test 4 –6 wkly until stable	ACB 2006
Sub-clinical hypothyroidism.	If not treated, monitor progression with 4 wkly TSH & FT4 until 16 – 20/40, then at least once again between 26 – 32/40.	Stagnaro-Green 2011
HbA1c: women with diabetes planning pregnancy.	Test monthly.	NICE CG63

REPRODUCTIVE HORMONES		
TEST	**MRI + ADVICE**	**REFS**
Progesterone day 21: ovulation-detection	If cycle irregular, test wkly from d21 until next period.	NICE CG156
FSH: ? menopause.	x2 tests, 4 – 8w apart.	Goodman 2011
Prolactin: ? drug-induced hyperprolactinaemia.	Stop Px for 3d then re-test.	Casanueva 2006 Melmed 2011
Prolactin: hyperprolactinaemia & starting dopamine agonist therapy.	Re-test prolactin after 1 month of Px.	Melmed 2011
Testosterone: ? deficiency in a man.	Re-test testosterone in a 9 am. sample to confirm.	Bhasin 2010
Testosterone: HRT monitoring.	• test 3 – 6 months after start. • then 3 – 4 monthly for first year. • See PSA	Bhasin 2010 Petak et al 2002
Oestradiol.	• IVF patients: test as often as daily. • Implant HRT: test before each implant re. tachyphylaxis.	

SPECIAL PROTEINS		
TEST	**MRI + ADVICE**	**REFS**
Paraproteins: asymptomatic myeloma	**3 months** initially.	www.bettertesting.org.uk Bird 2013
Paraprotein band <15g/L & no features of plasma cell dyscrasia eg. anaemia, pathological fracture or bone-pain, immuno-suppression, renal impairment.	**Annual** serum protein electrophoresis & paraprotein quantitation.	www.bettertesting.org.uk
MGUS	**Annual**	www.bettertesting.org.uk
Immunoglobulin replacement.	**3 monthly or less**, trough Ig & LFT tests.	UKPIN 2011
Immunoglobulins.	**6 months minimum** for other purposes. See above.	ACB 2013
Myeloma patients on active Px.	See local guidelines.	ACB 2013
CRP	**Not < 24 hr** after initial request except in children.	Hutton 2009

THYROID

TEST	MRI + ADVICE	REFS
TSH: screening asymptomatic people.	**3 y**	ACB 2013
TSH + **FT4:** Hyperthyroidism	• **Radioiodine or thyroidectomy**: Test **4 – 6 wkly** post-treatment, then 3 monthly for up to 1y & then annually if biochemically euthyroid & stable. Life-long surveillance is required for the development of hypothyroidism or recurrence of hyperthyroidism. • **Thionamidides**: **4 – 6w**. Test 3 monthly once maintenance dose achieved. • '**Block & replace'**: **4 – 6w**. Then test 6 monthly once maintenance dose achieved,	ACB 2006
TSH: hypothyroidism	• **2 months**. After starting T4 Px or changing the dose, because it takes this long to equilibrate. • **Annually**. When stabilised on long-term T4 Px. • Test **FT4 at least annually** in patients with **secondary hypothyroidism** stabilised on T4 Px	ACB 2006
TSH, FT4 + **FT3**: sub-clinical hyperthyroidism, monitoring adults	• If TSH is below the ref range but > 0.1 mU/L, re-test TSH, FT4 + FT3 with a **3 – 6 month MRI** (less if patient is elderly or has CVD eg. 1 – 2 months) after excluding non-thyroidal illness & drug interference. • **6–12 monthly,** test TSH & FT4 if treatment is not started,	ACB 2006
TSH + **FT4**: monitoring sub-clinical hypothyroidism in adults.	Confirm sub-clinical hypothyroidism in **3 – 6 months** to exclude transient ↑ TSH. If anti TPO Abs +ve, check TSH & FT4 annually. If –ve, check 3 yearly.	ACB 2006

TUMOUR MARKERS

TEST	MRI + ADVICE	REFS
AFP: hepatocellular carcinoma (HCC) surveillance & screening patients at hi risk.	**6 months**	Ryder 2003 NACB 2010
AFP: monitoring HCC recurrence	**3 – 6 months**.	NACB 2010
AFP: acute response to Px. Going ↑ or ↓ ?	**1 w** Equilibrium = 5 x $t_{½}$ = **4w**	
CA125: screening if ↑ FH of ca. ovary	**1 y**.	NACB 2010
CA125: detection & early management of ca. ovary.	Retest CA125 in < 1 month when imaging is – ve.	NICE CG122
CA125: monitoring recurrence.	**1 month**.	Duffy 2013
CA15.3: ..	**2 months**.	NACB 2008
CA199: ..	**1 month**.	ACB 2013
CEA: ..	**2 – 3 months**.	EGTM 2013. NACB 2009
β-hCG: tumour marker: molar pregnancy after evacuation	• Test **weekly** until in ref range. ↑ t½ ie. > 3d suggests residual disease. • then monthly for 1st year.	Bidart 1999
β-hCG: non-molar tumour marker: after resection.	↑ t½ ie. > 3 d suggests residual tumour & correlates inversely with survival. hCG may ↑ with chemotherapy & tumour lysis.	Bidart 1999
β-hCG: tumour marker, general point	If the rate of change in hCG alters, get an **urgent repeat** to confirm the result.	NACB 2009
PSA: screening.	When first result is raised, repeat in 6w to assess trend.	PCRMP 2010
PSA: monitoring neoplastic disease.	• 3 monthly for first 1 – 2 yrs. • 6 monthly for 2 yr. • then annually.	NACB 2008

U + E (urea, creatinine, Na + K)		
TEST	**MRI & ADVICE**	**REFS**
U+E: monitoring of stable inpatient.	**4 d** eg. an inpatient with an admission sodium in the ref range should not have it re-assayed within an average stay of 4d.	ACB 2013
U+E: ditto: on iv fluids (adults & paed.).	**1 d**	GAIN 2010
U+E: symptomatic patients or after hypertonic saline.	**2 – 4 h**.	GAIN 2010
U+E: AKI.	Check on admission & within 24 h.	UKRA 2011
U+E: ACEI & ARB Px.	**1 w**. Test before, 1 – 2 w after starting Px & after each dose change. Test more often in the elderly or patients with renal disease, disorders affecting electrolyte status or patients on drugs e.g. diuretics, spironolactone. Then 6 monthly or annually if stable. More often if renal function ↓.	CKS 2010
U+E: diuretic Px.	……*ditto*……e.g. ACEIs, ARBs, spironolactone. Then…*ditto*……	CKS 2010
U+E: monitoring in digoxin Px.	**8 d** After start or change in digoxin Px &/or change in interacting drug. Then test annually if no change.	UKMI 2002 CKS 2010
U+E: monitoring in digoxin + diuretic.	Regular monitoring.	CKS 2010