

Pathology News

May 2008 - ACB Focus Special Edition

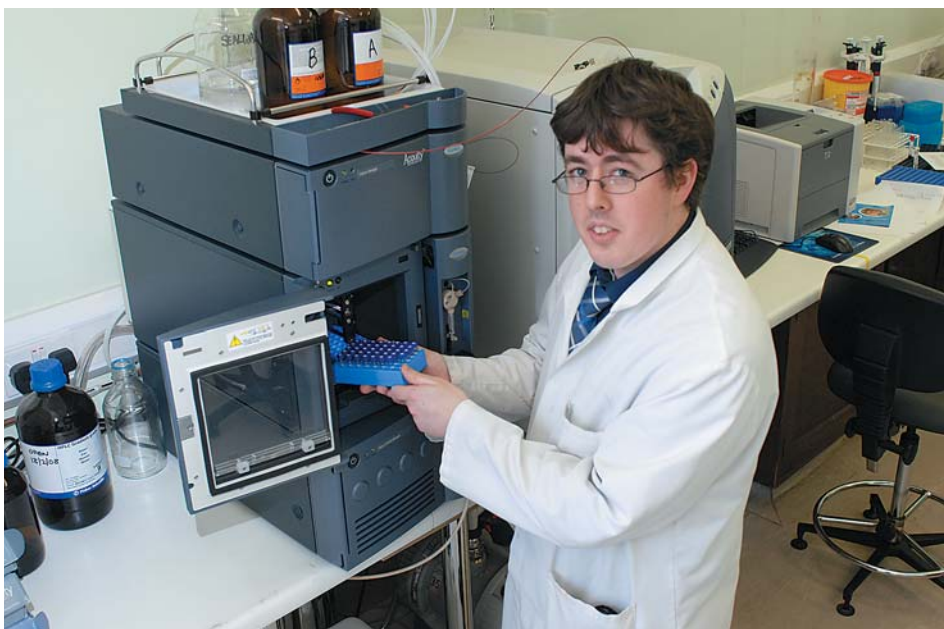
Providing a service - not just an assay

This issue includes audit information for users as well as important news on advances in the services that we offer.

We are building on the success of our stand at the IBMS last September with a presence at Focus 2008. This gives us an opportunity to meet users and gain feedback. If you are visiting Focus then do come along and see us. We are also running a series of mini-seminars at lunchtime that may be of particular interest to trainees.

25-Hydroxyvitamin D: three for one offer!

We are pleased to announce that our new 25-Hydroxyvitamin D assay came into routine use on 1st May. This is based on the latest LC-MS/MS methods and means that we now offer total 25-Hydroxyvitamin D, D₂ and D₃ results to our users. This is a significant advance and together with an emphasis on clinical support and relevant R&D we hope will contribute to better treatment of Vitamin D associated disease.



Philip loads up the vitamin D LC-MS/MS

**ACB Focus 2008:
our stand is No.3**

Located in
the main
Exhibition
Hall



DON'T MISS

**Mini-seminars
on our stand**

12.45 Tuesday

Vitamin D: 3 in one go!

12.45 Wednesday

**When and why to
measure Manganese**

12.45 Thursday

TPMT to die for ...

13.15 each day

**A pain-free way of
Passing the MRCPath
Practical**

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TPMT ... emphasis on service

The TPMT assay at City Hospital was introduced in August 2004 and since then our service has been in continuous development.

Thiopurine drugs suppress the immune system and are used by different disciplines including gastroenterology, dermatology and respiratory medicine. There are several new thiopurine drugs currently being trialed around the world and our assay is being used to help in this work.

The TPMT enzyme metabolises thiopurine drugs to inactive metabolites. The standard dose given to patients is higher to counteract this loss. Genomic expression of TPMT shows pharmacogenomic variability.

Around 1:300 of the population have no enzyme activity. For these individuals a standard dose of thiopurines represents a significant overdose; normally an alternative drug treatment is considered.

A further 10% of the population are heterozygote for TPMT gene expression with reduced activity. For these patients a reduced dose regime is used and here the measurement of the active metabolites, thioguanine nucleotides, (6-TGN's) is increasingly seen to be beneficial for dose adjustment and increased drug efficacy, and we also now offer this test.

Phenotyping and genotyping ... a combined approach

Our strategy for a clinical TPMT service is to use both TPMT genotyping and phenotyping.

Phenotyping rapidly determines the TPMT activity status of all patients. For genotyping we use a multiplex amplification refractory mutation system (ARMS) strategy to screen for the common TPMT mutations TPMT*2 and TPMT*3, which in Caucasian, Asian and African-American populations are responsible for about 95% of all mutant alleles for deficient TPMT activity.

When do we do DNA analysis?

Deficient results: all deficient results are confirmed by genotyping. Users receive the results of this investigation with a clear interpretation.

Transfusion and adverse reactions: there is a possibility that the true TPMT phenotype is masked by the donor blood. All requests that indicate the patient has received blood products within 120 days undergo genotyping. If there is any discrepancy between the phenotyping and genotyping results the requester is notified. Genotyping is also performed for patients who have previously undergone a serious adverse reaction to

thiopurine drug treatment.

Ongoing audit: Concordance between low TPMT activity results and heterozygous genotype as part of our internal QC of the assay. The genotyping from these studies is not sent back to users.

We do not make any additional charge for this DNA analysis – it's all part of our service! However we do not accept specific requests for TPMT DNA analysis only. If you require TPMT genotyping only then please contact us.

Performance audit 2007 - 08

Total number of requests:	14,007
Number of deficient (<6)	44 (0.3%)
Number of low (6-34)	1630 (11.6%)
Number of normal (35-79)	12,022 (85.8%)
Number of high (80 plus)	311 (2.2%)
[TPMT activity nmol/6-MTG/g Hb/hr]	
Number of users:	175
Mean turnaround time:	42.5 hours (including weekends)
Number of samples rejected:	Incorrect sample received (serum) 31
	Mismatch between sample and request form details 28
Number of user logs	157 (1.1% of workload). NB All were for requests for results not received.
Analysis could not be performed due to technical/staffing problems	0 days



Shaista runs the TPMTs

Dry Faecal Elastase

It has been six months since we introduced our improved FE-1 service which includes a dry method for analysis of wet stool samples that can't be analysed by the conventional method.

Our first audit of what difference this has made is shown opposite. The new assay has meant a significant reduction in the need for repeat faecal sampling.

Method used:	Conventional (April 06 - March 07)	Conventional + dry (October 07 - March 08)
Unable to give a result or commented 'interpret with caution'	18%	0%
Total number of samples analysed	2033	1283
% of samples analysed using dry method	-	11%

Blood Manganese analysis

Manganese is an essential trace element that serves as a catalyst for enzymes including hydrolases, kinases, decarboxylases and transferases. It is also required for the activity of metalloenzymes for example arginase, and mitochondrial superoxide dismutase.

Sources of manganese include: whole grains, nuts, vegetables and teas. However, bioavailability may be affected by the presence of other substances in the diet, such as tannins, oxalates, phytates and fibre.

When to monitor manganese

Deficiency features include dermatitis, hypocholesterolemia and weight loss. Patients on long-term parenteral nutrition are particularly susceptible to deficiency if not supplemented.

Toxicity

Due to its low absorption and rapid elimination by the liver, toxicity is rare. Effects include neurological and psychiatric disorders. Patients with cholestasis and/or small bowel resection on Home Parenteral Nutrition are at most risk of accumulating toxic levels of manganese. Intoxication can also occur through inhalation.

Specimen requirements

A whole blood sample collected in a Sodium Heparin tube. Blood samples are prone to contamination if taken via stainless steel needles, hence, a plastic canula should be used for sample collection or the first few mls of blood discarded.



Aleha in the trace elements laboratory

FT IR stones service overcomes diagnostic waiting times

A key pressure on laboratories over the last year has been the emphasis on ensuring all pathology tests are undertaken within the new directive on working times. Historically renal and bladder stones have tended to have long turn rounds. We are now in the second year of offering our stone analysis service, which includes supplying a photographic record for the patient's notes as well as the latest fourier transform infra-red analysis.

25-Hydroxyvitamin D - why the change?

Increasingly in the UK both vitamin D₂ and D₃ preparations are being used to treat patients with vitamin D deficiency.

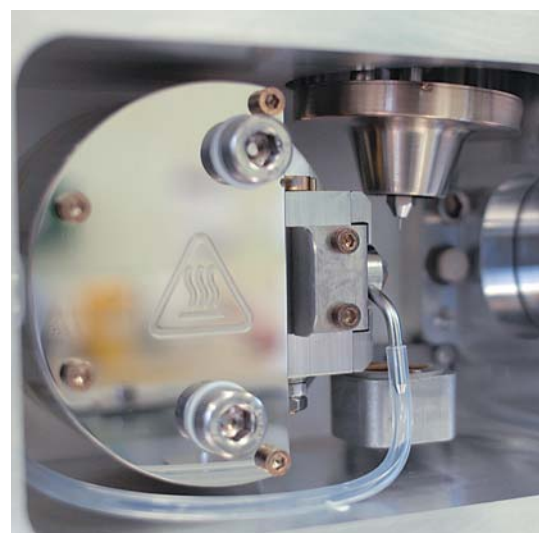
Recent evidence has now shown that vitamin D₂ potency is less than one third that of vitamin D₃, and its duration of action is much lower. This may result in patients treated with D₂ responding slower than expected to treatment. Therefore it is clinically important especially for monitoring patients treated for vitamin D deficiency (rather than just screening) both 25-hydroxyvitamin D₂ and D₃ are measured.

Our previous RIA method for measuring 25-hydroxyvitamin D was not able to measure D₂ and D₃ separately, and also under detected D₂. To offer the best service to our users we have greatly improved our 25-hydroxyvitamin D service by developing a new LC-MS/MS method for measuring vitamin D₂ and D₃ separately, which we report in addition to the total 25-hydroxyvitamin D level.

Recognising the increased sensitivity of our new method we have also introduced a new reference range for severely deficient 25-hydroxyvitamin D.

Our new service

We use a fast crash liquid-liquid extraction method and measure 25-hydroxyvitamin D₂ and D₃ separately using a highly sensitive LC-MS/MS system, the Waters Quattro Premier XE MS detector with ACQUITY Ultra Performance LC. It includes a 26,27



The electrospray is at the heart of the new D LC-MS/MS analysis.

-hexadeuterium-25-hydroxy Vitamin D₃ internal standard.

Sample requirement:
Minimum 0.25 ml serum or plasma

Turnround:
2 working days

Reference Ranges:
Total 25-Hydroxyvitamin D (µg/L)
Deficiency < 10
Severe Deficiency < 6

We also report the individual values for 25-hydroxyvitamin D₂ & D₃ in µg/L

When there is significant D₂ present (>60% of the total 25-hydroxyvitamin D) the following text comment will be added to reports:

'Significant ergocalciferol (vitamin D₂) present'

You can of course download the user information leaflet from our website and it will also be available at our Focus 2008 stand.



Vitamin A, E, D and Carotenes

Sample handling and transport

We often receive enquiries from users on the best way to store and transport samples for our fat-soluble vitamin services.

Our own studies have shown that vitamins A, E and D show excellent stability at room temperature and exposure to light, with concentrations decreasing after 14 days by less than 9%. Carotenes are less stable and will decrease regardless of the conditions of storage.

We would therefore recommend to all our users that:

- There is no need to protect samples from sunlight
- Samples are stored at 4°C prior to shipment
- Samples are sent as soon as possible by first class post at ambient temperature.

Monitoring Ethylene Glycol exposure

Ethylene glycol is a common additive of antifreeze and screen wash. It is highly toxic, and as little as 30 mL can be lethal to adults.

Ethylene glycol is a colourless, odourless, syrupy liquid with a sweet taste, and children and animals can sometimes accidentally consume large quantities. It is also involved in cases of attempted suicide.

Ethylene glycol poisoning should be suspected in an intoxicated patient with a high anion gap and a high osmolal gap may also be present. Other laboratory features include hypocalcaemia, urinary crystals, and a non-toxic blood alcohol concentration.

Treatment should not be delayed until acidosis indicates the development of severe toxicity. Optimum management requires urgent measurement of plasma

ethylene glycol, typically in conjunction with methanol, and ethanol measurement to monitor antidotal treatment.

The City Hospital Toxicology Ethylene Glycol Service uses gas chromatographic techniques to distinguish between the alcohols, and for the measurement of ethylene glycol. Turnround is 3 hours and the service is available 24 hours.



Specialist assays

Offered by SWBH NHS Trust Pathology Department

Biochemistry	Turnround	Cost
Vitamin A	3 days	£11
Vitamin D (Total, D2 and D3)	2 days	£19
Vitamin E	3 days	£11
Carotenes	5 days	£26
TPMT Service	2 days	£29
Thioguanine nucleotides (TGNs)	3 days	£29
Faecal elastase-1 service	3 days	£29
ACE	3 days	£12
Stone Analysis	5 days	£23
Toxicology	Turnround	Cost
Ethylene glycol	Available 24 hours	£77
Manganese	3 days	£25
Haematology	Turnround	Cost
P50	1 day	£130
Erythropoietin	3 days	£27
Microbiology	Turnround	Cost
Daptomycin	3 days	£30

Contact point

Department	Email	Telephone
General	info@cityassays.org.uk	0121 507 4233
Biochemistry	biochem@cityassays.org.uk	0121 507 5353
Haematology	haem@cityassays.org.uk	0121 507 4241
Microbiology	micro@cityassays.org.uk	0121 507 4228
Trace elements	elements@cityassays.org.uk	0121 507 4135
Toxicology	toxicology@cityassays.org.uk	0121 507 4135

cityassays.org.uk

Our website gives basic details for many of the tests that we offer to other laboratories.

This includes:

- Downloadable PDF files of the user information leaflets
- Relevant information and background details for the tests
- Up to date details on the turnaround time for the tests that we offer.

