Posters to be displayed on Tuesday

11
Evaluation of the Randox full range C reactive protein assay on the Abbott Aeroset system

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The reference interval for C reactive protein (CRP) is approximately 0-6 mg/L with concentrations of 10-40 mg/L associated with mild inflammation and concentrations of 40-200 mg/L found in acute inflammation and bacterial infection. Elevated CRP levels within the 0-6 mg/L interval have been implicated in cardiovascular morbidity and mortality and serial CRP levels within this range are also useful in early neonatal sepsis as a guide to antibiotic therapy. A recently developed CRP assay (Randox Laboratories, Crumlin, N Ireland) with a reported range of 0-160 mg/L has been adapted for use on our high throughput automated analyser, Abbott Aeroset.

At mean concentrations of 2.18, 4.89 and 22.10 mg/L, intra-assay imprecision was 1.10%, 0.91%, 1.01%, n=20. Between-assay imprecision was 1.82%, 1.85%, (n=95) at mean concentrations of 2.18, 4.93 mg/L and 2.38% at a mean of 21.50 mg/L (n=70). Linearity assessed by comparison of triplicate assays of a serially diluted control and comparison of expected and measured results was 1.6-172 mg/L. Detection limit determined by the mean of 20 replicates of saline plus 2 SD of 20 replicates of the lowest concentration calibrator was 0.05 mg/L. Functional sensitivity assessed by analysis of 10 replicates of 5 patient specimens diluted to near the expected limit of detection range showed %CVs of 12.2, 11.2, 13.9, 17.7, 28.1 at means of 0.30, 0.25, 0.18, 0.10, 0.09 mg/L respectively. Functional sensitivity is therefore around 0.10 mg/L Duplicates of 12 NEQUAS samples showed the % mean bias from the all laboratory trimmed mean ranged from (-2.5%) at a mean concentration of 1.2 mg/L to (-0.92%) at 134.1 mg/L with a maximum on one control of 19.5% at a concentration of 15.9 mg/L.

CRP in the clinically useful range can be measured quickly and reliably on the Aeroset analyser within and outside normal working hours.

12
Alkaline phosphatase isoenzyme analysis: Sebia Hydrasys and Beckman Paragon agarose electrophoretic methods compared

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Aim: To compare serum alkaline phosphatase (ALP) isoenzyme level (% of total ALP activity) using two agarose gel electrophoretic methods.

Methods: 64 serum samples were analysed on the Sebia Hydrasys semi-automated and Beckman Paragon manual electrophoretic systems. Densitometric analysis was carried out using Sebia Phoresis scanning software and an Appraise Densitometer for each system respectively.

Results: Significant positive correlations between % isoenzyme measured by each system were found for bone, total liver and intestinal isoenzymes (liver r=0.75, bone r=0.53, intestinal r=0.90). No correlation was found between macromolecular ALP on the Beckman system compared to Liver 2 fraction using the Sebia method, this fraction being detected in 2/64 samples by the Beckman method and 58/64 on the Sebia system. When % isoenzyme results were compared to manufacturers reference ranges for each method, the Sebia system showed greater sensitivity for detecting levels above the upper limit of the reference range for liver ALP (Sebia 18/64, Beckman 7/64) and levels below the lower limit of the range for bone ALP (Sebia 13/64, Beckman 9/64) than the Beckman system. For intestinal ALP, the Beckman system showed greater sensitivity for detecting levels greater than the upper limit of the range than the Sebia method (Sebia 21/64, Beckman 27/64).

Conclusions: The % results for liver, bone and intestinal ALP assayed by each method are comparable. However, the Sebia system is exquisitely more sensitive to the presence of Liver 2/macromolecular ALP. Comparison of results with method dependent reference ranges however, indicates that these are not directly comparable between the systems. Being semi-automated, the Sebia system is more robust than the Beckman system. Gels run on the Beckman system can be interpreted qualitatively whereas densitometry is mandatory for interpretation of Sebia gels.

13
Establishing a referral service for thiopurine S-methyltransferase phenotyping

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We have developed a new procedure using 6-thioguanine for the determination of thiopurine S-methyltransferase (TPMT) activity in erythrocytes to identify patients at risk of thiopurine drug toxicity. All steps are undertaken in a sealed tube and heat is used to stop the TPMT reaction, removing the need for solvent extraction prior to HPLC analysis of the enzyme product, 6-methyl thioguanine. This assay has been CE marked, making it the first such in-house assay in use in an NHS laboratory. We have established reference intervals for

TPMT activity in 1,000 outpatients in the largest prospective study of TPMT activity undertaken.

The assay shows excellent precision with a within-batch and between-batch imprecision giving a coefficient of variation of 2.9% (mean = 41.5 nmol 6-MTG/gHb/hour, n=10) and 7.3% (mean = 41.0 nmol 6-MTG/gHb/hour, n=16), respectively.

The distribution of TPMT activity in 1,000 individuals studied was found to be trimodal, ranging from ≤1 to 76 nmol 6-MTG/gHb/hour, with a mean activity of 33 nmol 6-MTG/gHb/hour. There was a significant difference ($p \leq 0.001$) found for the mean TPMT activity between white Europeans (35 nmol 6-MTG/gHb/hour, n=456) and Afro-Caribbeans (30 nmol 6-MTG/gHb/hour, n=180).

Six individuals had deficient TPMT activity ≤2 nmol 6-MTG/gHb/hour. The homozygote status for deficient patients identified by our method has been confirmed by DNA analysis.

We have offered a service to others for TPMT activity for six months. During this time we have phenotyped 410 patients from 31 NHS trusts and have identified 3 deficient patients. We have designed a medical alert card for deficient patients to show their clinician. We are working with UK NEQAS on an international QA scheme for TPMT phenotyping. Our approach offers a cost-effective phenotyping service with a fast turnround, suitable for a regional or national referral center.

14
Comparison of thiopurine S-methyltransferase activity using different methods for preparing red blood cell lysates

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Clinicians are increasingly aware of the advantages of testing thiopurine S-methyltransferase (TPMT) activity prior to commencing thiopurine drugs to identify patients at risk of thiopurine induced toxicity. This is leading to a greater clinical demand for TPMT testing.

We have developed an assay for the determination of TPMT activity in red blood cells (RBC), which involves several steps: preparation of a RBC lysate, enzyme incubation, heat treatment to inactivate the enzyme and precipitate proteins, and HPLC analysis of the reaction product.

The use of whole blood as the starting point for the enzyme assay has been investigated to simplify the pre-incubation steps and speed up analysis. Three types of RBC lysate were prepared from EDTA blood, a whole blood lysate, a dipped lysate of packed RBC taken from the tube bottom after centrifugation (avoiding the white cells and plasma) and a reference lysate of RBC washed in physiological saline.

The protein content of the supernatant after heat treatment reduced to <0.05 g/L using the dipped and reference lysate and 0.4 g/L in the whole blood lysate. The whole blood lysate method was therefore considered unsuitable for HPLC analysis as too much protein would be injected onto the analytical column.

The imprecision of the method using dipped lysate had a within-batch CV 4.1% compared to a CV 3.8% for the reference lysate method (n=10, TPMT activity 25 nmol 6-MTG/gHb/hour). There is a methodological difference between the two procedures and for 43 patient samples the dipped method results were 8% higher than the conventionally produced lysates, the regression line being $y = 0.99X + 0.70$.

The use of a dipped lysate for TPMT analysis simplifies sample preparation, speeding up turn-round. It also facilitates primary tube sampling for measuring RBC TPMT activity, which could in the future be incorporated into an automated system.

15
Erythrocyte thiopurine S-methyltransferase stability under different environmental conditions; recommendations for appropriate transport and storage

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Thiopurine S-methyltransferase (TPMT) activity is used to assess patients prior to thiopurine drug treatment to minimise toxicity and optimise effectiveness. TPMT exhibits genetic variation, giving rise to a trimodal distribution, with 0.3% of the population lacking the enzyme and a further 11% having low activity. Screening to detect these patient groups prior to treatment means serious side effects can be averted. Changes in TPMT activity under different conditions have been studied so that guidance can be issued for appropriate sample transport and storage.

A 30 mL blood sample was collected from a healthy volunteer, aliquoted into 0.5 mL EDTA plastic tubes and stored at 4°C, room temperature and 37°C. A reference lysate was prepared at time zero, aliquoted and stored at 70°C. TPMT activity was determined at timed intervals using the conversion of 6-thioguanine to 6-methyl thioguanine (6-MTG), with HPLC measurement of the 6-MTG produced using our recently published method.

For storage at 4°C the enzyme showed excellent stability with no change in TPMT activity even after 271 h. At ambient conditions the TPMT activity fell by 20% after 168 h. At 37°C the enzyme was much less stable exhibiting a reduction in activity of over 50% by 30 hours. It should be noted that the experiment was conducted in July in a non-air conditioned laboratory with midday temperatures up to 33°C.

It is important that pre-analytical changes are understood and appropriately controlled so that TPMT activity reflects patient status. This study shows that samples for TPMT analysis can be transported in a postal system for up to 96 h with minimal loss of activity in an EDTA blood sample, and without pre-treatment or special transport conditions. It is possible to store samples at 4°C for at least eleven days prior to analysis without compromising the results.

16
Interference by haemolysis in the Dade Behring Dimension RxL creatinine assay

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Dade Behring report no significant interference by haemolysis in their modified kinetic Jaffe creatinine assay up to a haemoglobin level of 0.5 g/dL and more recently up to 1 g/dL.

Whilst reviewing daily cumulative U&E reports, it became apparent that this was not the case. Serum samples with visible evidence of haemolysis showed not only the expected increase in potassium, but also a reciprocal decrease in creatinine.

This decrease in creatinine ranged from 17 to 45% at levels of creatinine within the reference range with a mean fall of 27%.

A small study was conducted in which one of a pair of blood samples was haemolysed to yield 13 g/dL haemoglobin. 50 µL of haemolysate was added to 1.0 mL serum providing a haemoglobin concentration of 0.65 g/dL. This sample was double diluted with serum down to 0.02 g/dL haemoglobin. The serum creatinine concentration was 115 µmol/L. Samples were assayed in duplicate. The in-house assay between batch imprecision is 3.4% (85 µmol/L), 1.6% (304 µmol/L) and 1.5% (523 µmol/L).

The curve showed a sigmoidal reduction in creatinine down to 76% of the expected concentration as the haemoglobin concentration increased to 0.65 g/dL.

This data confirms the negative interference by haemolysis on serum creatinine values by this method.

17
The role of pH and phosphate concentrations in affecting urinary calcium and magnesium precipitation

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In the analysis of urinary calcium (Ca) and magnesium (Mg), to avoid their precipitation, it is recommended to collect urine into an acid preservative or acidify after collection. However, little attention has been paid to the conditions of urine that affects this precipitation. We have therefore investigated the role of pH and phosphate concentration and established whether a large molar excess of phosphate could reduce precipitation. This was also modelled using known dissociation constants in simulated urine. 10 mL aliquots were taken from 24 hr urines collected from 21 patients and adjusted to cover the pH range 2.0-10.0. Ca, Mg and phosphate were then measured on the Roche P-module (Roche Diagnostics, Lewes, East Sussex, UK) at different pH levels. As an index of precipitation, a reduction of >20% compared to the original unadjusted urine sample was considered significant. The mean (standard deviation) for Ca, Mg and phosphate concentrations (mmol/L) were 2.2 (1.64), 1.5 (0.76) and 10.2 (4.50) respectively. At pH >6.5, 28 of 63 samples (44%) had a fall in Mg and 23 of 59 (39%) had a fall in Ca >20%. There was no relationship between these changes and phosphate concentrations nor with pH. In the pH range 2.0-6.5 none of the samples showed any significant change in Ca or Mg concentrations. Using the thermodynamic modelling program (JESS, Murdoch University, Australia) precipitation of candidate compounds, brushite ($CaHPO_4.2H_2O$), commenced above pH 5.5 with a maximum at pH 6.5 and struvite ($MgNH_4PO_4.6H_2O$) precipitated at pH 6.0 with a maximum at pH 7.2 and were not reduced by 20-fold molar excess of phosphate.

In conclusion, Ca and Mg show a tendency to precipitate in urine (possibly as insoluble phosphates) when the pH is greater than 6.5. However, this was not related to the molar excess of phosphate and may be more dependent on other variables such as citrate or ionic strength. For routine purposes it is recommended that urine pH is measured and acidification be undertaken if necessary before analysis.

18
Evaluation of a harmonised Roche liquid based bicarbonate reagent kit on Integra and Roche Modular-P platforms

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A new harmonised bicarbonate liquid reagent (Roche Diagnostics) for Roche Modular-P and Integra systems was evaluated. The assay is based on a NADH coupled reaction with measurement at 340 nm. The aims of the study were to confirm the analytical performance of the bicarbonate liquid reagent and the reliability of the reagent under routine conditions.

Serum bicarbonate was measured using Roche Modular and Integra automated analytical platforms.

The evaluation used both commercial control materials and patient samples.

Within-day imprecision studies showed CVs of <2.5% (Modular-P) and <1% (Integra) for control materials covering the range 16-31 mmol/L. Between-day studies showed greater imprecision of 5% for both instruments. At lower levels imprecision was seen to markedly increase, both within and between day.

Deming regression shows the following comparisons with the routine laboratory method for bicarbonate (Modular-P BioMerieux, Cat. No 6 1652 or Roche Integra, Cat. No 0766763) with the new harmonised reagent (HR) system

A: HR-Modular-P = 0.8448 BioMerieux (r^2=0.929)

B: HR-Integra = 0.9268 Integra 0.5545 (r^2=0.925)

Interference due to icterus and lipaemia was within acceptable limits on both platforms. Interference by haemolysis was acceptable on the Modular-P but not on the Integra.

The liquid-based reagents show acceptable performance for routine measurement of bicarbonate on both analytical platforms.

19
A simple method for the analysis of urinary sucralose in the investigation of intestinal permeability

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A novel technique for the site-specific assessment of intestinal barrier function has recently been described in animals. We have modified this technique for use in humans. The resultant triple sugar permeability test requires the analysis of urinary sucralose, a recently developed artificial sweetener, following oral administration. The aim of this study was to develop a simple method for urinary sucralose analysis using high pressure liquid chromatography.

Sample preparation involved addition of 100 µL of phenyl-β-D-glucopyranoside 10 mg/mL (internal standard) to 10 mL of urine, which was then passed through a 0.45 µm syringe filter. Separation was performed using a reverse-phase C18 column. The mobile phase comprised an isocratic water:methanol mix (70:30), at a flow rate of 1 mL/min. Injection volume was 100 µL. Detection was with a refractive index detector, and integration based upon peak areas. All samples were assayed in duplicate. Aqueous and urine-based calibration curves were produced, and sixty standards made by adding sucralose (25-100 mg/L) to the urine of randomly selected hospital inpatients. The percentage recovery of sucralose in each standard was used to calculate inter-assay variability.

The calibration curve obtained by plotting sucralose concentration against peak area was linear in the concentration range 10 to 500 mg/L (r^2=0.99). Mean recovery of sucralose in 60 urine-based standards was 103.1% (CV 6.07%). The lower limit of detection was 10 mg/L.

The method described represents a simple assay for urinary sucralose, which has acceptable accuracy and imprecision. In combination with standard dual sugar probe techniques, the quantification of urinary sucralose excretion may permit the non-invasive assessment of colonic and small-intestinal barrier function in human subjects.

20
Is homocysteine stable in dried blood spots?

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Neonatal screening for homocystinuria is not widely performed by homocysteine analysis in dried blood spots (DBS) partly due to the uncertainty about sample stability as homocysteine levels rise in whole blood *in vitro*. Some centres screen for homocystinuria by measurement of methionine, however this misses >20% of cases.

The aim of this study was to adapt an in-house plasma homocysteine assay for analysis in DBS and to determine the stability of DBS homocysteine.

Blood was collected into K-EDTA tubes (n=30) and spotted onto Guthrie cards. Samples were centrifuged, plasma removed and frozen. Hcy was eluted from Guthrie cards in a solution of cysteamine (internal standard) and tributyl phosphine in dimethyl formamide for 30 minutes. The elutant was analysed for total homocysteine by HPLC with fluorescence detection using SBDF derivatisation. DBS homocysteine was measured at day 0 (baseline), 1, 2, 3, 7, 14 and 28. Baseline DBS results were compared to plasma results.

The CV of the DBS homocysteine method was 5% (within-batch), 8% (between-batch). The method was linear up to 140 µmol/L and had a lower limit of detection of 4 µmol/L. There was good correlation between baseline DBS and plasma results (r=0.8014). DBS homocysteine results were as follows: baseline, 14.5±4.7 (mean±SD); day 1, 14.0±4.6; day 3, 13.4±4.5; day 14, 13.3±4.6, day 28, 13.2±4.7. Homocysteine was stable in DBS up to 24 hours (p=0.17, paired t-test) followed by a small consistent reduction to 7% by day 3. The rate of reduction was then more modest, a total fall in homocysteine of 9% at day 28.

We have described a method for the measurement of DBS homocysteine which is reproducible, robust and is suitable for the detection of elevated homocysteine concentrations found in homocystinuria in samples stored for up to one month.

21
Development and validation of an improved isocratic HPLC method for the measurement of essential fatty acids in erythrocytes

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The body can synthesise all the fatty acids except for linoleic and alpha:linolenic acids. These essential fatty acids can only be obtained from dietary sources. Red cells are a potential store of fatty acids (FAs). Measurement of the essential fatty acid content of erythrocytes can be used as an index of nutritional health status of subjects and to detect essential FA deficiency. We therefore developed an improved isocratic reversed phase HPLC method to measure erythrocyte FA concentrations in adults and neonates.

The aim of the study was to determine if the measurement of the fatty acid content of erythrocytes could be used as an indicator of the nutritional and health status.

Venous blood (2.5 mL) from adult subjects (n=10) was collected in EDTA-containing tubes. The remaining erythrocytes were washed in cold physiological saline. Washed red blood cells (erythrocytes) were counted before haemolysis, hydrolysis, extraction, evaporation and derivatization with 4-bromomethul-7-methoxycoumarin. Fatty acid concentrations (arachidonic, palmitoleic, linoleic, eicosatrienoic, oleic, palmitic and stearic acids) were measured by an isocratic reversed phase HPLC method using fluorimetric detection, after separation on a C-8 Spherisorb column.

For all measured erythrocyte fatty acids, linearity of the method is maintained between 50-100 μL of red blood cells. The mean within-batch CV of the method was 7% and the mean between-batch CV was less than 15%. Analytical recovery ranged from 80-134%. The respective correlation coefficient, slope ($x10^{-3}$), and x-intercept for each fatty acid were as follows: arachidonic 0.9988, 3.0, 5.2; palmitoleic 0.9975, 2.4, 7.5; linoleic 0.9989, 2.5, 5.2; oleic 0.9969, 2.2, -11.9; palmitic 0.9980, 2.1, -7.0; stearic 0.9984, 1.5, -2.1; and eicosatrienoic 0.9973, 2.5, 6.8.

As the assay was developed to measure FAs in erythrocytes from neonates, sample size was very important.

We found that using more than 100 μL of suspension red cells was wasteful and, in some cases actually reduced FA sensitivity.

Provided careful measures are taken, this isocratic HPLC method is acceptable for clinical use to measure erythrocyte FAs in low volume samples from neonates

22
ProANP in canine heart disease: method evaluation and determination of optimal collection medium for Guildhay's Canine ProANP 31-67 ELISA

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In humans, heart failure is predominantly due to left ventricular dysfunction, whereas in canines the cause is often primary atrio-ventricular insufficiency. ProANP has emerged as a potential candidate to aid clinicians when differentiating between symptoms due to cardiac disease and those with non-cardiac causes.

ProANP is the precursor molecule of ANP; which is released from atrial myocardium under stretch, or at increased pressure. More stable in the circulation than ANP, proANP is degraded into 3 fragments; 1-30, 31-67 and 79-98. The 31-67 fragment shows complete homology between humans and canines, and an ELISA originally developed for use in humans has been shown to correlate well with clinical and radiological diagnoses of heart failure in canines.

This study aimed to compare results using 3 collection media commonly employed in veterinary clinical pathology; EDTA, lithium heparin and serum gel tubes. Recovery, the occurrence or non-occurrence of proteolysis, dilutional parallelism, and the correlation between results were some of the parameters considered.

The methodology employed a competitive ELISA with a solid phase sheep antibody specific for human proANP 31-67. Unlabelled peptide in standards, quality control or patient samples competes with a biotinylated peptide (tracer) for the limited number of sheep antibody binding sites. After washing, and incubation with peroxidase conjugated streptavidin, further washing to remove free conjugate enables the bound peroxidase to lyse substrate added, producing colour; its intensity being inversely proportional to the amount of proANP present.

Recovery and dilutional parallelism were shown to be best with serum. Proteolysis was excluded and no separation measures were required for up to 48 hours after taking samples. This enables posting of whole blood samples from veterinary practices. Good correlation was seen between samples taken into EDTA and those into serum gel tubes, allowing establishment of reference ranges for dogs with and without cardiac failure.

23
The comparison of urinary free and total methylated catecholamines with free catecholamines in the laboratory assessment of catecholamine secreting tumours

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The measurement of urinary total methylated catecholamines (mets) is increasingly being advocated as the most reliable way to detect catecholamine secreting tumours. It has recently been shown that urinary free mets are an equally useful alternative. The automated system ASTED for catecholamine measurement can readily be modified to measure both free catecholamines and the free normetadrenaline (fNMA) and metadrenaline (fMA). We have therefore evaluated the robustness of this system for the routine analysis of urine (n=40 from 32 patients) free compounds (nmol/24 hr) with previously documented increases in individual total mets (µmol/24 hr) reported as median, range and normal values.

An increase in 27 urines of total normetadrenaline (tNMA, 6.8; 4.2-48.9; <4.0) was associated with increases in 26 of either free noradrenaline (fNA, 1425; 957-15048; <590) or fNMA (1271; 747-12996; <650). An increase in fNA alone was seen in 5 and in 2 a rise in only fNMA.

An increase in 12 urines for both tNMA (51.8; 7.1-579.2) and total metadrenaline (tMA, 9.4; 2.7-132; <2.0) was associated with increases (n=6) in both fNA (8246; 4746-45680) and free adrenaline (fAD, 5107; 437-15171; <190). The free mets were increased in 11 samples, fNMA (18101; 1501-97590) and fMA (3920; 890-39605; <350). In one only fNA (903) was raised.

In a separate urine an increase in total metadrenaline (3.7) was associated with increased fMA (1593) but not fAD (49).

We have demonstrated that the ASTED™ system can reliably resolve both free catecholamines and their free individual methyl derivatives during a single run.

In comparison with elevations in total mets, abnormal values for urinary free mets and/or catecholamines were consistently found. It is recommended that measurement of both free catecholamines and free methylated compounds be performed to ensure that abnormalities associated with catecholamine secreting tumours are detected.

24
Unreliability of testosterone measurements in adult females

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There is currently much concern on the overall reliability of methods used for measuring circulating testosterone concentrations in female patients. More specifically we had suspicions that false positive results were obtained using the Bayer Immuno-1 analyser. Samples from females in which elevated testosterone concentrations were obtained using this analyser were therefore subjected to further analyses.

We identified elevated (>3.0 nmol/L) Immuno-1 testosterone results in 151 samples over a 12 month period, where testosterone was within the reference range using the Vitros ECi immunoassay (upper limit of normal [ULN] for this assay being 2 nmol/L). Sample volume was sufficient to repeat measurements by the ADVIA Centaur immunoassay (ULN 3.0 nmol/L). For a limited number of samples (n=52) further measurement was obtained by an in-house extraction radioimmunoassay (ULN 3.2 nmol/L).

The median and range of testosterone measurements were 3.5 (3-15.8), 2.4 (0.3-4.0) and 1.6 (0.1-2.0) nmol/L for the Immuno-1, Centaur and ECi methods respectively. For this selected group of patients poor correlation between the extraction method and the Immuno-1 method was obtained (r=-0.17), whereas some degree of correlation between the extraction method and the Centaur method was observed (r = 0.68). Furthermore, in 10 patients with Immuno-1 testosterone levels that were elevated to a degree that is suggestive of significant underlying pathology (>5 nmo/L), all were within the reference range by the Centaur method.

Our results indicate that the Immuno-1 procedure produces a significant number of false positive results which could lead to either misdiagnosis or protracted unnecessary investigations. In light of the current findings we now routinely confirm any elevated testosterone result obtained by our routine Immuno-1 analyser at Edinburgh Royal Infirmary on a locally available ECi instrument, and in the event of any disagreement a further measurement is performed on a Centaur analyser based in Glasgow.

25
Modified method for extracted assay for female testosterone measurement on the Abbott Architect analyser

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Some samples from female subjects give falsely high results when measured in direct immunoassays. We

have modified a method described previously (Kane J W, Layton T, *Proc ACB National Meeting* 2002) for the extraction and measurement of female testosterone, by substituting the commercially available testosterone diluent with calf serum. This has resulted in significant cost savings to the laboratory (calf serum £0.07 per mL vs. commercial diluent £26.33 per mL).

Briefly, 300 mL of serum is extracted with 3ml ether by vortex mixing for 4 minutes. The aqueous layer is frozen and the ether layer decanted into 75 x 12 mm borosilicate glass tubes. The ether is evaporated in a 45°C water bath and the dried extract reconstituted with 300 mL calf serum (Sigma product no. C9676). After vortex mixing testosterone is measured on the Abbott Architect analyser following the normal protocol.

Mean recovery of 4 levels (1, 2, 5, 10 nmol/L) of added testosterone from female serum samples was 92% (range 75-99%). The within batch precision of the extracted method assessed as the difference between duplicate measurements was 6.7% (n=25). The interassay precision (n=73) was 10.5% at a testosterone concentration of 3.0 nmol/L. Extracted testosterone values on female samples (n=73) ranged from 33% to 143% of the unextracted value (mean 68.8%).

We are currently extracting all female samples prior to testosterone measurement. Our assay performance is comparable to that previously described but is significantly cheaper to run.

26
Immunometric determination of serum testosterone in females

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Cross-reaction of antibodies used in the measurement of testosterone with testosterone metabolites has been reported. The aim of this study was to determine whether this phenomenon was evident in measurements of serum testosterone in women using the Roche E170 automated immunoassay method.

Serum testosterone was measured using the Roche E170 automated immunoassay method before and after a diethyl-ether extraction step. The same samples were also assayed in parallel using DPC's Coat-a-count radioimmunoassay method.

Serum measurements of testosterone in females (range 0.7-15.2 nmol/L) by E170 method were significantly lower after diethyl-ether extraction (mean percentage of pre-extraction measurement, 55.5±24.1%, n=51, $p<0.001$) and not significantly correlated ($r^2=0.65$) whereas testosterone measurements by the Coat-a-count method were not significantly different after diethyl-ether extraction (85.6±18.3%, n=51) and were correlated ($r^2=0.95$). Serum measurements of testosterone in females by the Coat-a-count assay were significantly lower than by the E170 method (mean percentage of E170 measurement, 66±22%, n=51, $p<0.01$) and the methods were not significantly correlated ($r^2=0.84$). The two methods were however, comparable and significantly correlated after the diethyl-ether extraction step (Coat-a-count measurement as mean percentage of E170 measurement, 108±38%, n=51, $r^2=0.97$).

Testosterone measurements were significantly lower by the DPC Coat-a-count method than by the Roche E170 method and the methods were not correlated. However after diethyl-ether extraction the methods were correlated suggesting that the specificity of testosterone measurement by either one or both of the methods was improved by the inclusion of the extraction step. Diethyl-ether extraction of samples caused a greater reduction in the testosterone measurement using the E170 method than for the Coat-a-count method.

27
Evaluation of the Roche E170 SHBG assay

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Measurement of SHBG is primarily used in combination with testosterone as an indicator of excessive androgenic action, where androgen levels are normal. By calculating the free androgen index from the ratio of total testosterone to SHBG, it is possible to determine the approximate amount of the biologically active free-testosterone. SHBG is measured within our department using the DPC-Immulite, the assay carried out as batches on a weekly basis and testosterone is measured daily using the Roche Modular-E170. Following the recently introduced SHBG assay for the E170, consolidation of both assays onto the E170 platform appeared attractive in that both results would be available within 24 hr, without the need for a separate aliquot.

Evaluation included comparison of 36 patient results using both Immulite and E170 assays. The ranges of results were 19.3-170.0 nmol/L and 17.6-168.7 nmol/L for Immulite and E170 respectively. Deming regression showed $y=1.02x + 1.07$ and paired t-testing ($p=0.842$)

showed no significant difference between the methods. Analysis of 12 NEQAS samples with results between 26.4 and 140.0 nmol/L, gave a small positive bias against the ALTM. Deming regression showed y=1.10x + 1.07. Imprecision was assessed at three levels; 17.2 nmol/L, 85.5 nmol/L and 169.0 nmol/L; the CVs were 1.9, 3.1 and 4.0% respectively. Linearity studies were satisfactory.

However, difficulties in sourcing a suitable QC material has, as yet, prevented us from switching to the E170 method for the measurement of SHBG.

28
Comparison of three 5-α-dihydrotestosterone ELISA kits

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The aim of the study was to select the most appropriate kit for the measurement of dihydrotestosterone (DHT) in hCG stimulated children suspected of 5-α-reductase deficiency. We compared the performance of three ELISA kits, namely BIO (Biosource, Quidel Diagnostics, Oxford), IBL (IBL Hamburg) and AD (Alpha Diagnostic, Autogen Bioclear UK Ltd). We examined 64 serum samples, 26 males plus 38 females, age 14.3±7.8 years (mean±SD), range 0.1-20.9 years. The ELISA methods were similar in procedure: 50 µL serum was incubated with 100 µL enzyme conjugate for one hour. After decanting and washing, 150 µL TMB substrate was added, incubated 15-30 minutes and the reaction stopped and read within 30-60 minutes at 450 nm.

Deeming regression was used throughout for the evaluation. The BIO assay was evaluated for inter-batch precision on two successive days with the same samples, n=34, range 170-2630 pmol/L. We found that BIO day 1 = 1.21 BIO day 2 + 2. Comparison of the same 34 samples with the other kits was: BIO = 1.15 IBL +81 and BIO = 1.05 AD +142. The BIOassay was not considered further due to its high crossreactivity (32%) with testosterone.

As the IBL and AD kits had identical crossreactivity with testosterone, (8.7%), further evaluation was carried out with a larger sample range. A single high result on the flat part of the standard curve was discrepant, (AD=4909, IBL=2231 pmol/L) and was trimmed from the correlation. The trimmed result was: IBL=0.94, AD+83, n=63, range 98-2940 pmol/L

Age-related reference inervals for DHT expressed as mean ±SE were determined for four age ranges as follows: 1) 0-5.0 years, (n=7), AD= 780±350, IBL=830±380; 2) 5.1-10.0 years, (n=10), AD=350±100, IBL=398±100; 3) 10.1-15.0 years, (n=15), AD=900±290, IBL=780±130 and 4) 15.1-20.0 years, (n=30), AD=1490±170, IBL= 1450±140.

We conclude that the AD and IBL DHT kits are very similar in performance. There is an increase in DHT associated with increasing age, however, further investigation is required to verify the apparent reversed trend observed in our first and second age groups.

29
Evaluation of the Roche Elecsys SHBG assay

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Roche launched a fully automated non-isotopic electrochemiluminescent SHBG assay for their Elecsys analysers in the UK in 2003. The assay has been calibrated against the first international SHBG standard (95/560). Analysis is carried out in 18 minutes and reagent packs are stable for between four and seven weeks once opened.

The analytical performance of the assay using an Elecsys 1010 analyser was assessed by determining the precision, linearity on dilution and by comparisons with patient and NEQAS samples.

The within-batch CVs at 17 nmol/L and 43 nmol/L were 0.81% (n=20) and 1.9% (n=20), respectively. The between-batch CVs at these concentrations were 1.5% (n=20) and 1.7% (n=20), respectively. Linearity on dilution was found to be good. There was no significant difference between values obtained on Becton-Dickinson SSTII Plus (gel) vacutainers and plain clotted tubes (n=20).

Results on patient samples were compared against the Ortho Diagnostics immunoradiometric assay at the Royal Victoria Infirmary in Newcastle, which performed well in the UK NEQAS SHBG scheme. Passing and Bablok regression analysis showed Elecsys 1010 = 1.20 Ortho + 0.5 (n=33). Comparison with ALTM values on NEQAS samples (n=8) showed a mean bias of +13.0%.

In summary, the Roche Elecsys SHBG assay is rapid, precise and convenient. The stability of the reagents would enable laboratories with a relatively low workload for this analyte to provide a cost effective in-house service.

30
Evaluation of automated immunoassays for the measurement of urinary free cortisol

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Twenty four-hour urine collections for analysis of cortisol are commonly used as the front-line investigation for Cushing's disease. Automated assays for measuring

urinary free cortisol (UFC) are known to have variable performance, however the lack of an easily accessible gold standard method means that their use is widespread. In a bid to move away from isotopic semi-automated analysis of UFC we have investigated the performance of several automated assays.

UFC levels were measured in 100 urine samples by five different methods namely Beckman Access 2 cortisol, Bayer ADVIA Centaur direct cortisol, Bayer ADVIA Centaur indirect cortisol, Roche E 170 cortisol and DPC coat-a-count. When pre-analytical extraction was required sample were processed according to manufacture's instructions with slight modification. Dried extracts were reconstituted in charcoal-stripped outdated definbrinated blood transfusion plasma.

Significant differences ($p<0.0001$) were seen between the results obtained following analysis using direct methods compared with indirect assays, with the results varying up to 3-fold between the various assays. This is likely to be due to the presence of interfering substances in urine.

Performance of the indirect assays was more in-line with the results obtained by the DPC coat-a-count method however comparisons between both direct automated assays and the DPC method showed significant differences ($p<0.05$). Results from the Bayer ADVIA Centaur method correlated well with those of the DPC assay. No relationship could be demonstrated between the Roche method and DPC.

This study demonstrates the variability in performance of automated assays for cortisol in the determination of UFC. Establishment of method-specific reference ranges is crucial to ensure clinical validity of UFC measurements.

31
Prednisolone measurement by liquid chromatography tandem mass spectrometry

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Bayer Award

Prednisolone is a corticosteroid that is often used for long term suppression of the immune system; for example in asthmatics and transplant recipients. With severe side effects including osteoporosis, diabetes mellitus and adrenal suppression it is appreciated that the dose should be kept as low as possible. The monitoring of prednisolone would allow the physician to tailor the dose to individuals requirements, assess compliance and absorption and improve the outcome of these patients. We have developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay for the measurement of prednisolone in serum or plasma.

Samples (500 µL) and deuterated (d6) prednisolone (internal standard) were extracted using Waters Oasis® HLB columns, the methanol eluant was dried down and the extracted prednisolone was reconstituted in 50:50 mobile phases. The samples were then transferred into a 96-deep well microtitre plate, of which 20 µL was injected into the LC-MS/MS system. A Waters Atlantis® column (3.0 mm x 50 mm) was eluted with a step gradient of 50% to 95% methanol containing 2 mmol/L ammonium acetate and 0.1% (v/v) formic acid, at 0.5 mL/min. The column was operated at ambient temperature.

The retention times were 2.75 min for prednisolone and 2.72 min for d6 prednisolone. Cycle time was 5 min. The transitions used were m/z 361.3>147.1 for prednisolone and m/z 367.2>150.3 for d6 prednisolone; monitored using a Quattro micromass spectrometer. The between-batch precision of the method was 8%, 4% and 5% at concentrations of 75 µg/L, 375 µg/L and 750 µg/L respectively. The within batch precision was <8% for the same concentrations. The lower limit of detection was 25 µg/L and the assay was linear to 4000 µg/L. There was negligible suppression of ionisation.

We have developed a robust assay for the measurement of prednisolone. This should allow easy monitoring of treatment in many patient groups, optimising prednisolone therapy.

32
Salivary cortisol assay using the Bayer Immuno 1

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Salivary cortisol is becoming increasingly popular in assessing adrenal function and investigating the effects of stress. Most reported results involve the use of kits intended for the assay of serum cortisol modified for measuring the lower concentrations found in saliva.

We have used solvent extraction to concentrate the salivary cortisol and, after redissolving in 4% BSA, have assayed the samples on the Bayer Immuno 1.

We have used the assay in a pilot study to access the effects of exercise on the concentrations of cortisol in saliva.

Fifteen volunteers aged 20-24 years collected 2 mL of saliva between 9-11am followed by a blood sample. The matched saliva and serum samples were assayed for cortisol. A further 15 volunteers collected 2 mL saliva before and after a sub-maximal exercise session on a treadmill.

At a mean concentration of 12.8 nmol/L the interassay CV was 7.18% and at 9 nmol/L the intra-assay CV was 7.03%. There was a significant positive correlation between the serum and salivary cortisol results ($r=0.757$ $p=0.003$)

In the exercise study saliva samples collected at rest had a mean of 15.7 (SEM 2.8) nmol/L. After 25 minutes exercise the mean was 19.6 (SEM 3.8) nmol/L. The t-test showed the difference between pre and post exercise to be not significant in comparing the results of all volunteers however within individuals 60% showed significant increases in salivary cortisol concentrations.

The concentration of cortisol in saliva by solvent extraction has been shown to be suitable for use on the Bayer Immuno 1 immunoassay analyser and could be measured on other similar automated systems. The assay has been used to assess the effect of exercise on cortisol concentrations and we hope to use it to further investigate the effect of other stress factors on cortisol secretion.

33
Evaluation of the effect of pH adjustment in the measurement of total urinary aldosterone

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Aldosterone-18-glucuronide, an important metabolite of aldosterone, requires deconjugation before measurement of total urinary aldosterone output (TUA). This is commonly achieved by hydrolysis at pH 1.0 and quantitation by radioimmunoassay (RIA) after extraction. Hydrolysis may be incomplete at pH 1.0, resulting in an underestimation of TUA levels since hydrolysis at pH 0.2 gives a much greater yield, although the lower pH degrades aldosterone. Unless acidification is performed accurately, the pH may drop below 1.0 and damage to free aldosterone may occur.

TUA was measured using an RIA Count-A-Count method from DPC (TKAL1/2). In the recommended method, a fixed amount (25 mL) of 3.2N HCl is added to 250 mL of urine to hydrolyse the glucuronide metabolite. Preliminary investigations showed that using these fixed volumes, the pH of the urine sample was consistently lowered to less than pH 1.0.

The aim of this investigation was to compare results obtained by accurately adjusting samples to pH 1.0 (±0.05) with those obtained following the hydrolysis method outlined in the DPC kit.

Mean values for TUA measured following the kit method and accurate pH adjustment were 38.8 and 42.1 pmol/L respectively. The two methods correlated well (r=0.93) and a clear relationship (pH1 method = 1.02(kit method) + 2.54) existed. A Bland-Altman bias plot demonstrated a significant high bias in the pH 1.0 hydrolysis method, particularly at lower values. This might suggest proportionally more aldosterone is damaged by the low pH when there is less aldosterone in the sample.

This investigation has shown that the pH adjustment carried out as described in the DPC kit insert consistently lowers the pH of urine aliquots below pH 1.0. This results in a mean lower recovery than when pH is more accurately adjusted to pH 1.0.

34
Macroprolactin determination: a methodological pitfall

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The clinical symptoms of menstrual irregularity, infertility and galactorrhoea in women and impotence and reduced libido in men are common. Measurement of serum prolactin is a key investigation to identify the minority of such patients who merit further investigation. However, increased serum prolactin can be due to the presence of macroprolactin which has reduced biological activity. Macroprolactinaemia is a frequent cause of misdiagnosis and mismanagement.

We perform PEG precipitation on all prolactins exceeding the upper limit of normal, unless previously checked, using a standard protocol. Serum prolactin is measured on a Bayer Immuno-1 auto-analyser. Hyperprolactinaemic samples with a PEG recovery of <60% are deemed to contain macroprolactin. An estimate of the bio-active monomeric prolactin is made and reported with a covering letter identifying the presence of macroprolactin.

In a 12 month period, 282 serum prolactin results above the reference range were identified, with 39 newly detected macroprolactinaemias. The finding of 4 samples with recoveries of <30% within a short space of time seemed suspicious and led to an examination of our PEG-precipitation protocol.

It was confirmed that the correct protocol was in use, however re-precipitation with fresh PEG reagent yielded prolactin recoveries of 100-105% for each of these samples.

We have thus established that the PEG reagent is not in fact stable for 3 months as originally suggested but should be prepared fresh each month. We now routinely measure the prolactin recovery on an IQC sample to check viability of the PEG reagent.

35
MCA-SAQSSE: a highly sensitive immunoassay technology for clinical biochemistry

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Immunoassay is the fundamental analytical technology relied upon for accurate diagnosis of disease. However, a

long-standing and widely-recognised need within the clinical biochemistry community is the attainment of greater sensitivity for the measurement of hormones, proteins and peptides to allow earlier, more accurate diagnosis and improved monitoring of treatment, which will result in improved prognosis. For many analytes, an increase in sensitivity of 100-1,000 fold above that which is currently available is sought.

To address this we have developed a novel technology, Melt Curve Analysis Single Analyte Quantitation through Single Strand Extension (MCA-SAQSSE) which is a marriage of traditional immunoassay and PCR melt-curve analysis. This new technology utilises direct labelling of the detector antibody with a small DNA molecule (the key). After formation of the antibody-protein-antibody complex, a large complementary DNA molecule is added (the lock) which engages the key , and as a combined lock and key can be specifically amplified by a palindromic primer and quantified using specific signal probes and melt-curve analysis.

We have applied this technology to the measurement of TSH in buffer and have observed at least a 400-fold increase in sensitivity with a 5% CV across the range of 0.00016-25 μIU/mL when compared with a HrP/TMB traditional format using common reagents. This technology offers the potential to significantly increase the sensitivity of both single-site immunoassays, two-site immunoassays and ligand assays and we are currently optimizing it for clinical use with human serum samples.

36
Evaluation of the Nichols Advantage vitamin D assay

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Vitamin D is essential to the control of calcium homeostasis and is mainly produced by exposure of the skin to sunlight. At latitudes in the UK synthesis occurs only between April and October. A little vitamin D is also obtained in the diet from dairy products and oily fish. Deficiencies in various disease states, ethnic groups and also due to dietary and lifestyle changes have resulted in an increasing demand for vitamin D assays. Automation of our vitamin D assay is highly desirable. We therefore compared the automated Nichols Advantage chemiluminescent assay(CIA) with the IDS (IRIA) and 'in house' (HRIA) manual radioimmunoassays which use radioiodine and tritiated labels respectively.

157 serum samples were assayed in parallel according to the appropriate protocols. Reproducibility was assessed using quality control data and dilution analysis was also performed.

Median sample values and ranges observed were 34.5 (7.75-122), 41(12-110) and 51 (<17-152) nmol/L for the HRIA, IRIA and CIA respectively. Corresponding reference intervals were 15-100, 23-113 and 25-170 nmol/L respectively. 54 samples were outside the range for the HRIA, 38 for the IRIA and 25 for the CIA. Precision studies demonstrated interassay CVs (n=10 in each case) of 19%, 9% and 15% at values of 11, 22 and 46 nmol/L for the HRIA, 5.8% and 9.4% at values of 27.6 and 106 nmol/L for the IRIA and 8.8%, 3.7% and 3.5% at vaues of 31, 62 and 148 nmol/L for the CIA. Linearity studies showed acceptable linearity between concentration and dilution in all assays with a marginally better performance from the CIA.

The automated CIA assay offers advantages in precision, turnaround time and cost and must therefore be considered for adoption.

37
Comparison of immunoreactive PTH assays and determining PTH reference range on Elecsys E170 (Roche)

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Parathyroid hormone (PTH) secreted by the parathyroid gland is a 84 amino acid molecule and is the predominant form in subjects with normal renal function. The determination of PTH is important in the assessment of metabolic and renal bone disease. Over the last few years a number of PTH assays have been established with different specificity due to immunoheterogenous nature of PTH. Here we report comparison of two major automated analysers PTH methods. Immulite 2000 (IM2K, DPC) PTH uses a monoclonal anti-PTH (44-84) antibody and a polyclonal anti-PTH (1-34) antibody. PTH antibody specificity information is not provided in the E170 PTH kit insert. We analysed 95 normal serum (age 18-60 yrs, 48 female, 47 male) samples on E170. 24 patient samples were analysed on both analysers for comparison. Biochemical analysis, alkaline phosphatase, calcium, albumin and creatinine were performed on Olympus AU 640. 25 hydroxy vitamin D (25(OH) D) was analysed using Diasorin kit. All the normal subjects' biochemistry results were within the reference range. 25(OH) D results indicated that the normal subjects were vitamin D sufficient (ranges 27-103 nmol/L). Pearson correlation was 0.99 and the linear equation was $y = 0.83x + 0.7$ for patients samples. However, the PTH results were significantly different between the two analysers using paired student t test ($p<0.05$). This is probably due to differences in the PTH antibody specificity. The reference range quoted by the manufacturer is higher and different from the one we obtained in normal subjects (1.6-6.9 and 0.6-3.5 pmol/L respectively). Roche manufacturers reference population were 300 apparently healthy

subjects with age greater than 15 yrs with no signs or symptoms of metabolic bone or other disease (especially calcium abnormality, malignancy renal failure). However, no vitamin D status is indicated. The differences in the reference range quoted by the manufacture from ours is probably due to different population and more importantly, to take into account renal function and vitamin D status. Thus, the interpretation for a given PTH result may be different depending which reference range is used. This study highlights the importance of determining individual laboratorys PTH reference ranges and to take into account vitamin D and renal status of the subject.

38
MHRA evaluation of eight testosterone assays

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We have, with the help of off-site laboratories, evaluated 8 testosterone assays used in UK laboratories. Particular attention was paid to the ability of the assays to measure low levels of testosterone.

Imprecision was assessed using four patient pools and Bio-Rad Liquichek control material. The pool with an all method mean value of 1.12 nmol/L gave a range of results between 0.58 and ≤1.73 nmol/L with between-assay CV of 14-30% compared to the pool with a mean of 11.45 nmol/L which gave a range of 9.88-13.33 nmol/L with between assay CV of 5-19%. Measurements made with Liquicheck material were not consistent with those made with patient pools which demonstrates the importance of using patient samples when assessing assay performance.

Recovery data shows that most methods have a recovery between 90%-105%. However, assay recovery does not appear to be an indicator of assay bias. Low recovery of 79.4% was not reflected in serum results, and good recovery of 98.4% gave a marked positive bias with the patient pools.

Accuracy was assessed using UK NEQAS samples. All methods showed some bias against the UK NEQAS all method mean at the low level (+60% to 50%) with some assays showing a positive bias at one level and a negative in another.

Our findings show that no method was without problems. This evaluation shows that some methods perform poorly with high imprecision and poor correlation; sensitivity is not good and there appears to be matrix interference with some methods. There is still much scope for manufacturers to improve sensitivity, precision and reliability.

39
Development of a fully automated application of the IDS OCTEIA 25-OH vitamin D ELISA for use in large population surveys

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Assessment of 25-hydroxy vitamin D status in population surveys has previously been laboriously undertaken using the Diasorin I^{125} radiolabeled method. This complex manual procedure necessitates organic solvent pre-extraction and shows considerable inter-analyst and inter-laboratory variability. The availability of direct, non-isotopic, extraction-free enzyme immunoassays presents the possibility of reducing variablity and labour through automation.

We have developed a fully automated application of the IDS 25-hydroxy vitamin D EIA Mk2 kit on the Dade-Behring BEP2000 analyser and assessed suitability for further use in the 1958 British Birth Cohort Survey.

Performance characteristics include imprecision 7.2%, 5.5%, 6.9% at Vit D levels of 16.1, 72.5 and 94.8 nmol/L respectively; functional sensitivity = 5.0 nmol/L and linearity up to at least 155 nmol/L. Passing and Bablock analysis of 142 samples from the early survey phase gave the relationship EIA = 0.80xDiasorin-1.2. Serum values showed good stability over 5 freeze-thaw cycles. The analyser configuration allows 4 x 96 well assay plates to be processed together and significant labour-saving benefits arise from the fact that only 11% of overall analytical process time is hands-on as opposed to 29% for the manual Diasorin method.

We conclude that although the automated IDS method results differ from those of the manual Diasorin method this relationship has been quantified and can be monitored for inter-survey data comparisons. The greater ease and overall consistency with which vitamin D can be measured with this automated ELISA application makes it ideally suited to analysis of large numbers of samples generated in population surveys.

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Increased instrument intelligence: can it reduce error?

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Error reduction in automated immunoassay systems is an important objective, often overlooked, in evaluations of different systems. This study assessed this capability in three different systems, the Ortho-Clinical Diagnostics VITROS® ECi Immunodiagnostic System, the Abbott AxSYM®, and the Roche Elecys® 2010 analyzers.

Materials and Methods: Three immunoassay analyzers were challenged by repeatedly assaying pooled human serum samples of unknown analyte concentrations over a three-week period. One of the three analyzers had an intelligent process for fluidics checks, Intellicheck™ Technology, including bubble detection. A group of samples was vortexed to create bubbles and immediately placed on each analyzer and assayed. A second group of samples consisted of good quality non-vortexed samples typical of those routinely analyzed in the laboratory. An error was defined as a result greater than three standard deviations below the mean of the analyte for the individual analyzer. Accuracy, precision and efficiency were measured and the error rate determined.

Results: The precision and accuracy were acceptable for all three analyzers, with the lowest CV% obtained by the two systems utilizing chemiluminescence technology. The two analyzers without bubble detection reported a combined total of 23 incorrect results from a total pool of 600 vortexed samples. The analyzer with bubble detection reported one specimen incorrectly. No errors were found in the non-vortexed specimens. No significant difference in overall process time was seen for any of the analyzers when tests were arranged in an optimal configuration.

Conclusions: The analyzer with advanced fluidic intelligence, Intellicheck™ Technology, demonstrated the greatest ability to appropriately deal with an incomplete sample aspiration by not processing and reporting a result for such a sample. This study shows that such pre-analytical process control could reduce errors due to this cause resulting in a reduction of misreported results.

41
Mind the gap! pseudohyponatraemia and the Roche Integra

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Pseudohyponatraemia is not of concern to laboratories using direct reading ion selective electrodes (ISEs). Many laboratories using indirect ISEs choose to neglect this important phenomenon which may lead to inappropriate patient care. The replacement of Ortho Diagnostics Vitros analysers with Roche Integras saw the change from direct ISE serum sodium measurements to an indirect ISE technique. This required a protocol to advise our users of the presence of pseudohyponatraemia to avoid inappropriate clinical interventions. The original protocol was based on the Integra evaluation correlation studies which highlighted a difference of 6 mmol/L between the Vitros direct ISE, and the Integra direct ISE. All samples with a sodium level of <125 mmol/L had direct ISE sodiums undertaken. A difference >6 mmol/L between the indirect and direct measurements was further investigated with total protein and triglyceride measurements. Where triglycerides were >5 mmol/L or total protein >90 g/L a comment was added to indicate the possibility that reported sodium levels were falsely low. In all other cases pseudohyponatraemia was recorded as being excluded. The protocol was revised in 2003 due the increasing number of equivocal results leading to some confusion amongst staff. Two further algorithms were produced. The first, based on multiples of the mean (MoM), compared each test method result with the relevant reference range. Under normal circumstances the difference between the MoMs would be minimal usually <0.01; any >0.01 would be investigated as above. Although this would be the most accurate method for determining differences between the MoMs practically this was difficult to adapt to the Apex computer system. An alternative approach was based on the mean differences (10 mmo/L) between the direct and indirect reference ranges quoted by Roche. Comparative studies of the original and revised protocols showed that, respectively, 175 (65%) and 28 (11%) of samples would require further action. The latter protocol was adopted as the most usable and appropriate, with a view for further development to incorporate calculations for analytical variation and random errors.

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A systematic approach to instrument evaluation tailored to the needs of an immunoglobulin assay service

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Quantitative estimation of immunoglobulin levels is part of the routine service provided by most NHS biochemistry laboratories, and the scope has widened with tests such as free light chains. Assessment of the suitability of any analyser is a complex issue, but challenges in the field of immunoglobulin estimations present a distinct situation. Our laboratory uses the Dade Behring BN ProSpec® analyser at present for this purpose. We used the Beckman Coulter Immage® system to develop an analyser assessment procedure for suitability of use in such a role.

We collected information on systematic approaches to biochemistry instrument selection from the literature, local protocols and conventional practice. Having formulated a broad approach encompassing a wide range of factors, we assessed the Beckman Coulter instrument for our immunoglobulin assay service. We then refined our evaluation protocol in light of those features that turned out to be particularly relevant to the protein laboratory.

Finally, we performed a conventional method comparison study between the two analysers.

We identified a large number of factors with the potential to influence the choice of analyser, and developed a decision tree to aid objective instrument evaluation for an immune protein service. Using this, we have demonstrated that the Beckman Coulter Immage is well suited to small-to-medium laboratories with a requirement for flexibility in the assays available on a single instrument. However, the requirements of a university teaching hospital laboratory made it less suitable as our primary analyser. The method comparisons (167 consecutive samples) showed good correlations for IgG (r^2=0.97) and IgA (r^2=0.97), but less so for IgM (r^2=0.86). Bland-Altman plots were also constructed.

Our approach allowed us to efficiently compare the Beckman Coulter Immage's strengths (e.g. user defined reagent flexibility) to our current needs and capabilities with the Dade Behring ProSpec. We emphasise the importance of a systematic approach to instrument evaluation and have demonstrated the feasibility of adapting such assessments to specific needs.

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Leptin concentrations correlate with weight loss but do not predict response to therapy in patients with chronic hepatitis C infection

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The current gold standard treatment for chronic hepatitis C infection is combination therapy with pegylated interferon and ribavirin. The hepatitis C virus (HCV) occurs with a number of genotypes. HCV of genotypes 1a or 1b is more resistant to treatment than those with other genotypes. Weight loss occurs in 50% of patients on combination therapy. Leptin is an adipose-derived hormone involved in the regulation of feeding and energy balance. Leptin has been shown to be positively correlated with hepatic steatosis and fibrosis. The aim of this study was to examine the relationship between leptin, BMI and response to combination therapy.

Twenty-six patients (17 male: 9 female; 11 genotype 1 versus 15 non-genotype 1) with chronic hepatitis C infection were treated with 24-48 weeks of pegylated interferon and ribavirin. BMI, serum leptin and ALT were measured at baseline and throughout treatment. These parameters were then compared between sustained viral responders (SVRs) and non-responders/relapsers (NRRs).

Nineteen patients were SVRs (6 genotype 1, 13 non-genotype 1). There was no difference between BMI, ALT and leptin at baseline between SVRs and NRRs. At the end of treatment, ALT was significantly lower in SVRs than NRRs (p=0.04). 21 patients lost weight during therapy and 18 of these had decreased leptin concentrations. Leptin concentrations strongly correlated with BMI throughout the study (r=0.8; p=0.005). There was a significant positive correlation between the change in serum leptin and the change in BMI that was not related to response.

The change in leptin concentrations correlated with weight loss during treatment regardless of response. This data suggests that leptin is related to changes in BMI as a result of therapy rather than having a role in response to therapy.

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An unusual case of hyperammonaemia

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An 11 year old male presented to casualty in a barely conscious state following a 12 hour history of vomiting. His ammonia on admission was 156 μmol/L. His past medical history is complex. Although born at term he was small for dates. As a neonate he had surgery to correct an atrioventricular septum defect. During his early childhood he was found to have bilateral reflux nephropathy with scarring and mild to moderate high frequency sensorineural hearing loss. He also required mitral valve replacement and subsequent warfarin therapy. At the age of 10 years he was diagnosed with attention deficit hyperactivity disorder. Currently he has severe behavioural problems with a mental age of 5 years. During this admission an EEG showed seizure activity and he was presumed to be in non-convulsive status, which was treated with lamotrigine and phenobarbitone. CSF cultures, microscopy and viral screens were negative and the child gradually improved over 7 days without further treatment. Routine biochemistry, including LFTs, was normal and haematology results were consistent with warfarin therapy. Ammonia was persistently raised in the range 57-156 μmol/L; inherited metabolic causes were excluded. Subsequently an allopurinol loading test revealed subnormal results suggesting inadequate allopurinol had reached the liver. Imaging revealed aberrant venous drainage of the gut. The extrahepatic portal vein was patent but there was a large vessel shunting blood along the lesser curvature of the stomach. It was not possible to demonstrate a patent portal vein within the liver. Presumably ammonia produced in the gastrointestinal tract is bypassing the liver, via the shunt, and entering the systemic circulation. It is not clear whether the portosystemic shunt is related to a previous portal vein thrombosis or whether it reflects a congenital abnormality. Whatever the underlying cause this is the most likely explanation of the persistent hyperammonaemia.

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Fructose 1,6-bisphosphatase as a marker of hepatocellular damage in liver transplantation

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Bayer Award

Aspartate amino transferase (AST) and alanine amino transferase (ALT) are used as markers of hepatocellular damage in liver transplantation but are insensitive to acute changes. When cellular damage does occur levels remain elevated and poorly reflect further damage. Fructose 1,6-bisphosphatase (FBPase) is a key enzyme in gluconeogenesis and due to its location in the cystol of the periportal liver cells it has been proposed as an alternative marker of hepatocellular damage. We evaluated FBPase against conventional liver enzymes in an experimental porcine transplant model.

Porcine donor livers were subjected to various periods of cold ischaemic injury, group I (n=4) 4 hours, group II (n=6) 1 hour and group III (n=4) 0 hours, post donor hepatectomy and prior to preservation by normothermic extracorporeal sanguineous machine perfusion. FBPase, AST and ALT levels were measured in perfusate samples taken from the circuit during machine preservation. FBPase was analysed enzymatically by monitoring NADPH production at 340nm as fructose 1,6-bisphosphate is converted to fructose 6-phosphate. AST and ALT were analysed using conventional methods.

FBPase levels began to increase slowly after reperfusion in all three groups, with the lowest levels being seen in group III which remained below 100 U/L. FBPase in groups I and II rose to around 150 U/L at 6 hrs preservation. A similar pattern was seen for both AST and ALT. However, at 8 hrs preservation the FBPase levels increased in group I to 550 U/L and 750 U/L at 12 and 20 hrs preservation respectively. This was not reflected in the AST and ALT results which both increased slowly. The increase in FBPase may be related to graft failure, shown by a decline in bile production, as FBPase rose some hours after bile production fell. Therefore FBPase has the potential to be a sensitive and useful marker of liver function in transplantation.

46
Are persistently abnormal alanine aminotransferase values investigated in Primary Care?

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Minor elevations of alanine aminotransferase (ALT) over 6 months can be indicative of chronic hepatic disease. While there are currently no national guidelines for the evaluation of persistently elevated ALT values in asymptomatic patients, a panel of serological tests including hepatitis B and C serology, auto-antibody screen, immunoglobulins and serum ferritin concentrations and an ultrasound scan of the upper abdomen, may be appropriate first line tests to detect the more common disorders.

It had been noted that large numbers of patients with apparently unexplained persistent ALT elevations were undergoing frequent repeat testing indicating uncertainty as to how to proceed with investigations. An audit was therefore undertaken to assess the extent of this problem.

All adult patients with ALT concentrations over twice the upper reference limit (n=270) were identified from liver function tests requested by General Practitioners between 1st May and 30th October 2003. Previous biochemical, virological, immunological and radiological investigations were noted. Outpatient attendances and in-patient stays were also noted. Patients with known liver disease and patients who had subsequently died were excluded from further analysis. ALT values had been raised for over 6 months in 36% of the remainder.

The mean duration of ALT elevation was 41 months. No investigations appear to have been performed in 36% and 64% had been partially investigated. Of these 42% had undergone hepatitis B serology and 28% hepatitis C serology. An autoimmune profile had been performed on 35%. Serum ferritin and immunoglobulin concentrations had been measured in 37% and 12% respectively. The most common investigation was an ultrasound scan, which had been requested in 58%.

In conclusion the audit has demonstrated that persistently abnormal ALT values may not be adequately investigated in Primary Care and suggests the need for guidelines.

47
Is there an advantage to dry compared to wet samples in the faecal elastase test?

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Faecal elastase is gaining an increasing role as a test of pancreatic exocrine function. It is non-invasive and has high sensitivity and specificity for moderate to severe pancreatic exocrine disease, compared to classical tests of pancreatic function. However, the sensitivity can be reduced in mild exocrine disease. One reason for this could be variability in water content of stool samples and dry samples might offer a better sample material.

We have designed a method for measuring faecal elastase in a dry faecal specimen. The procedure has been used to compare results from the conventional wet

faecal assay with the new assay in 100 patient samples sent to us for faecal elastase determination.

Each sample was split and 100 mg measured wet weight. For the dry sample 100 mg faeces was collected onto a mesh support, dried at 40°C for 2 hours and then assayed in the same manner as the conventional sample. An internal control sample was run with each batch of samples in order to assess the between-batch variation of the assay.

Within-batch imprecision was CV=7.7% (x = 438 μg/g, n=10) for the wet method and CV=5.5% (x=905 μg/g, n=10) for the dry weight procedure. For the wet samples the patient mean was 380 μg/g compared to 679 μg/g for the dry samples. Of the 100 patients 18 wet and 13 dry samples were in the severe insufficiency range (≤100 μg/g); 3 dry samples giving lower values than wet. Using Deming linear regression the correlation coefficient was found to be r=0.88.

Faecal elastase measured in dried faecal material showed slightly better precision characteristics and results correlated well with the conventional wet faecal procedure. However there is no compelling evidence in this study that dry faeces should be analysed instead of wet faeces.

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Faecal occult chronic pancreatitis

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The analysis of human faecal pancreatic elastase -1 is of proven use in the diagnosis of chronic pancreatitis. However, the variation in faecal composition could be such that intra-individual variation of the test might be significant. In this study we have analysed faecal samples sent for faecal occult blood determination to investigate daily variation in elastase.

Faecal occult blood samples were stored at 4°C prior to analysis. The triplet samples, taken on three consecutive days, were analysed on the same analytical run to minimise methodological variation. Pancreatic elastase was analysed using the ELISA technique (Schebo Biotech), with each sample analysed in duplicate. A quality control material gave within batch imprecision CV of 7.7% (x=438 μg/g, n=10) and between-batch imprecision CV of 11.7 (x=480 μg/g, n=10)

Results for the triple patient-matched samples showed that faecal pancreatic elastase gave reasonable agreement, with a three-way regression analysis, giving r= 0.845, r=0.933 and r=0.933.

An unexpected finding was that in two of the twenty patients we found levels of pancreatic elastase clearly in the severe exocrine insufficiency range (<100 μg/g). One patient had levels averaging 13 μg/g faeces and a second levels averaging 59 μg/g faeces.

In our group of 20 patients we have shown a good agreement for faecal pancreatic elastase from different stool collections. The unexpected finding of two patients with low levels merits further investigation as neither of these had been previously diagnosed as having chronic pancreatitis. We postulate that faecal occult blood requests self-select for patients with a much higher prevalence of chronic pancreatitis than the normal population. If this is the case then screening samples sent for occult blood analysis could be an effective way of identifying patients with chronic pancreatitis, a rare but eminently treatable disease.

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The effect of different intravenous colloids administered during abdominal aortic surgery on endotoxin release and gastric perfusion

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Major surgery is associated with gut ischaemia, a fall in intramucosal pH (pHi) and translocation of endotoxin from the gut into the circulation. Endotoxin release has been implicated in the development of systemic inflammatory response syndrome (SIRS) and later organ failures. Recent studies suggest that the type of intravenous colloid used during surgery influences gut perfusion. This study aimed to compare the effects of abdominal aortic aneurysm (AAA) surgery on gastric perfusion and endotoxin release in patients given three different colloids.

Sixty patients were randomised to receive either 6% hydroxyethyl Starch (HES) with a mean Mwt of 200 kDa, 6% HES mean Mwt 130 kDa or 4% gelatine mean Mwt 30kDa. Endotoxin release was assessed by measurement of plasma core endotoxin antibody (EndoCab). During endotoxaemia, EndoCab concentrations fall due to binding with endotoxin. IgG EndoCab levels were measured by ELISA, pre and postoperatively up to 72 hours and expressed as the EndoCab to total plasma IgG ratio, to correct for protein leakage due to changes in vascular permeability. Gastric tonometry was used to measure the mucosal pHi as a marker of gastric perfusion during surgery and up to 72 hours postoperatively.

In the gelatine group median (95% CI) plasma EndoCab/IgG ratios pre op and 12 h post op were 17.7 (11.3-23.0) and 14.0 (10.7-17.1) units/g IgG (p=0.0006). There was no change in EndoCab/IgG ratios for the two HES patient groups. Ten minutes and 2 hours following clamp release, gastric pHi was higher in the HES 200 group compared with the gelatine group, (p=0.023 and 0.0002 respectively) and at 2 hours post clamp release was significantly higher in the HES 130 group (p=0.003).

During AAA surgery only patients given gelatine showed evidence of endotoxaemia, and both HES groups showed better gastric perfusion compared with gelatine. The use of HES during AAA surgery may preserve gastric perfusion and prevent endotoxaemia.

50
An HPLC-PAD method for the simultaneous measurement of mannitol, rhamnose, lactulose and sucralose in the assessment of whole bowel permeability

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Sucralose, a chlorinated derivative of sucrose can now be co-administered with lactulose, mannitol (or rhamnose) to give an assessment of whole bowel permeability. To date urinary sucralose has had to be assayed independently from the other small bowel permeability markers. We therefore aimed to develop an HPLC-PAD method to enable anaylsis of multiple markers within one chromatograhic run.

Urine is diluted between 1:5 and 1:20 with deionised water, depending on initial volume. Internal standard (melibiose 100 mg/L) is added and the mixture is desalted with ion exchange resin (Amberlite 120 H and IRA 402 Cl). Forty microlitres of supernatent is injected onto a Dionex PA-1 ion-exchange column. Probe molecules are eluted with a base eluant of 100 mM NaOH with a linear sodium acetate gradient of 0 to 75 mM over a period of 5 to 20 mins at a flow rate of 1 mL/min. Total run time is 25 mins including five minutes for column re-equlibration at the end of each run. Detection is pulsed amperometric using a gold electrode, without the need for post-column addition of NaOH. All sugar probes are eluted within 20 mins and there is no significant interference from other disaccharides, in particular lactose and sucrose. Sensitivity of detection for sucralose is 5 mg/L and lactulose 0.5 mg/L. The method is linear to 500 mg/L for all sugars. Analytical recovery is in the range of 90 to 105% and coefficients of variation range from 1.8% to 6.1%.

This technique allows the simultaneous measurement of a number of sugar probe molecules used in the assessment of whole bowel permeability.

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Early decline in serum IgA-tissue transglutaminase antibody levels after commencing a gluten free diet

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Previous reports have shown that IgA-tissue transglutaminase antibody levels (TGA) are correlated to the duration of a gluten free diet (GFD) and may therefore provide an objective measure of compliance with a GFD. This study provides evidence that TGA can be used as an early indication of response to and/or compliance with a GFD.

Data from 26 adults diagnosed with coeliac disease between June 2002 and December 2002 were evaluated. All had raised TGA at diagnosis. Compliance with a GFD was assessed by dietetic consultation and TGA measured 5-17 weeks and 3-6 months after commencing a GFD. Serum TGA was measured using the Celikey assay (Pharmacia Diagnostics).

Eighteen subjects were judged to comply strictly with the GFD; their TGA declined over 5-17 weeks with an apparent mean half-life of 4.4 weeks (sd=3.0). 8 patients did not strictly comply with the GFD; of these, 2 (non-compliant) had half-lives of 42 and 34 weeks and 6 (moderately compliant) had half-lives of 3-12 weeks. TGA levels continued to decline in those adhering to a strict GFD and 9 of 14 had levels <5 units/mL at 3-9 months after commencing a GFD.

TGA levels decrease rapidly (50% decline in 4.4 weeks) in patients complying with a GFD. This non-invasive test can provide additional objective evidence within a few weeks of commencing a GFD to support dietary assessment of compliance.

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IgA antibodies to human tissue transglutaminase: a first line test for coeliac disease in children

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It is important to have sensitive and specific serological tests for coeliac disease (CD) in children to avoid unnecessary biopsy. Here we report an audit of the effectiveness of tissue transglutaminase antibody (TGA) compared to endomysial antibody (EMA) for the diagnosis of CD in children.

Sixteen months retrospective audit, from June 2003, using TGA (Pharmacia Diagnostics) and EMA (The Binding Site) as initial investigations for CD in children (10 years and less) was performed. Serology and biopsy results were cross-referenced to clinical data.

TGA was available for 310 children. 288 had TGA <3.0 units/mL; 22 (7.1%) were ≥3.0 units/mL. 4 were in the range 3.0-5.0 units/mL; 2 of these were biopsy positive for CD and 2 clinically were not CD (no biopsy). 2 were in the range 5.1-10.0 units/mL, 1 had a positive biopsy, one is awaiting biopsy. In 16 children TGA was >10 units/mL: 13 of these have been diagnosed as CD (6 biopsy positive), 1 awaits biopsy; 2 patients are diabetics under follow-up but not clinically felt to have CD

at present (no biopsy). Excluding patients awaiting biopsy, specificity of TGA is 98.6% and 99.3% at >3 or >5 units/mL. Sensitivity of TGA for CD at >3 and >5 units/mL is 100% and 88.8% respectively. The positive predictive values are 0.80 and 0.89 at 3 and 5 units/mL.

Our experience confirms TGA as an effective first line test for the diagnosis of CD in children with comparable diagnostic accuracy to EMA. We suggest follow up and biopsy when TGA is greater than 3 units/mL.

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Use of red-cell thiamin diphosphate measurement to validate a screening instrument for detection of acute alcohol-associated thiamin deficiency

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The importance of early treatment of acute thiamin deficiency in alcohol misuse has been emphasised in a recent report from the Royal College of Physicians (2001). Parenteral thiamin is the treatment of choice, though a 'treat-all' approach has clinical (risk of anaphylaxis) and cost implications. The early recognition of at-risk patients is therefore of importance. We report here the evaluation of a Thiamin Deficiency Questionaire (TDQ), which together with clinical markers has been tested against measurement of red cell thiamin diphosphate (RBCTDP) as an indicator of thiamin deficiency.

Fifty-eight patients (36M, 22F, age range 21-65y) admitted with a history of severe alcohol dependence were studied. Blood samples were taken on admission and after 6 doses of parenteral thiamin (6 x 250 mg thiamin hydrochloride) over 48h, and analysed for RBCTDP (by HPLC) and other biochemical and haematological analytes, including gamma-glutamyl transferase (GGT). A TDQ of 15 items (7 nutritional, 5 alcohol use, 3 clinical examination) was completed, together with BMI measurement and Michigan Alcohol Screening Test (MAST) scoring.

RBCTDP concentrations were 95-274 nmol/L (range), mean 169.8, on admission and 120-604, mean 257.2, post-treatment. 31 patients (53.4%) had a RBCTDP below the reference range on admission. Compared with RBCTDP, a combination of all variables had a positive predictive value of thiamin deficiency of 83.7% (95% CI, 72.2-95.1%), and that of the TDQ alone 73.7% (95% CI, 60.3-87.0%). The strongest single identifiers were MAST score and GGT.

In cases where direct thiamin measurements are unavailable, or unavailable in a time frame to influence the clinical decision, this screening model that combines socio-demographic, clinical and biochemical factors, and standardised questionaire may enable early recognition of thiamin deficiency.

54
Comparison of direct and indirect methods for measuring riboflavin and their use in assessing riboflavin status in TPN patients

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Riboflavin (vitamin B2) is an important cofactor for various enzymatic reactions, and is an essential component of a healthy balanced diet. Riboflavin is hydrolysed to form flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are involved in the transfer of electrons in oxidation-reduction reactions. The primary aim of this study was to compare a direct high performance liquid chromatography (HPLC) method with an indirect enzymatic method that measures erythrocyte glutathione reductase (EGR) activity, in order to assess riboflavin levels. A normal reference range was established for both methods, and the most appropriate method used to assess the prevalence of riboflavin deficiency in patients receiving total parenteral nutrition (TPN).

Blood samples were obtained from 90 healthy volunteers in order to establish reference ranges for both methods. Blood samples were also obtained from 51 patients receiving TPN. The results obtained from the reference range samples for each method were compared using Spearmans Rank correlation. The results from the reference group and patients receiving TPN were compared using the Mann-Whitney U test.

The EGR activity coefficients were found to correlate well with the flavin levels measured by HPLC ($p<0.001$). The HPLC method was chosen for the analysis of the TPN patients mainly due to its increased sensitivity and resistance to interference compared to the EGR assay. There was no significant difference between the FAD and FMN levels in controls and TPN patients, except in the case of riboflavin, where TPN patients were found to have significantly higher levels than controls ($p<0.001$).

In conclusion, the HPLC method evaluated in this study was found to be superior to the popular EGR assay for assessing riboflavin status. Riboflavin deficiency is not prevalent in patients receiving total parenteral nutrition.

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Nutritional rickets among children in Peshawar, NWFP, Pakistan

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Rickets or ricket like features have been found to be caused or cured by a wide variety of nutritional factors. It seems that people with rickets are seldom screened for nutritional deficiencies. It is a common feature of the developing countries. In the developed world lack of sunlight and consumption of too little milk are contributing to a resurgence of rickets. A total of 164 children under 4 years of age, either sex, belonging to urban and rural areas were included. Diagnosed children were analysed for their serum calcium, phosphorus and alkaline phosphatase along with age, height and weight. Moreover, the effect of sunlight exposure, weaning diet and breast feeding was also studied.

Almost all children were deficient in calcium and phosphorus while alkaline phosphatase was above the normal. Children who were not exposed to sunlight nor breast fed but who were receiving the weaning diet, residing in multi-storied houses all showed radiological symptoms (98%). Children exposed to sunlight, but breast fed and receiving the weaning diet showed similar radiologial signs (97%). Perhaps the weaning diet was deficient (inhibitory). Moreover, in children who were only breast fed, radiological symptoms were less (48%) than the aforementioned two groups. In conclusion, access to sunlight, supplementation of milk or milk products, avoiding phytate containing vegetables and habit of breast feeding are recommended.

56
Serum cortisol and growth hormone levels in infants with marasmus

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Objectives: to assess serum cortisol and growth hormone levels in infants with marasmus.

Design: case series study in Al-Khansa Hospital in Mosul, Iraq.

Patients and methods: serum cortisol and growth hormone levels were measured in 24 infants with marasmus before the institution of nutritional rehabilitation and the results were compared with 20 infants with no nutritional problems.

Results: a significant increase in serum cortisol ($p<0.01$) and growth hormone ($p<0.001$) was observed. A significant correlation was noted between serum and cortisol and growth hormone ($p<0.001$) and between cortisol and weight: ($p<0.02$). No significant correlation, however, was reported between growth hormone and weight, between cortisol and weight deficit or between growth hormone and weight deficit.

Conclusion: A change in serum cortisol and growth hormone in infants with marasmus is noted. The exaggerated hormonal response may represent an adaptation of the body to the decreased protein and energy supply.

57
Is antioxidant response dependent on cell type?

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Oxidative stress is defined as an imbalance between pro-oxidant versus antioxidant species resulting in oxidative damage to biological macromolecules. The purpose of our study was to demonstrate the response of the cells towards free radicals (FR), once they are treated with different anti-oxidants (AO). Also to determine the optimal concentration of each AO which is effective in suppressing the production of FR.

Cells selected were RAW (macrophage) and FtO2B (hepatoma cell line) from rat tissue. These cells were treated with different concentrations of antioxidants; ascorbic acid (25, 50, 100, 250, 500 μmol/L), α-tocopherol (5,25,50 and 100 μmol/L) and selenium (0.1, 0.5, 1.5 and 2.5 μmol/L) acutely, 24 hourly and daily treatment for a week. The oxidative stress in our *in vitro* study was induced by iron catalysed ethanol generation of hydroxyl ethyl radical, which was quantified by EPR/ spin resonance technique

FtO2B and RAW cells showed significant production of FR at concentration of 500 μmol/L when treated daily for a week. RAW cells when treated with selenium at concentration of 1.5 and 2.5 μmol/L for a week showed increased production of FR. At the rest of the concentrations, FtO2B and RAW cells showed significant FR suppression with acute, 24hour and week treatment with α-tocopherol,selenium and ascorbic acid.

Antioxidant effectiveness appears to depend on the cell type being studied and the duration of their administration. The long-term pro-oxidant effect of selenium at high doses administration on phagocytic cells and ascorbic acid on both phagocytic as well as parenchymal cells requires further study to elucidate the mechanism

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Elevated serum methylmalonic acid in renal failure falls following transplantation

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Serum methylmalonic acid (MMA) has been advocated as a marker of B12 deficiency. Vitamin B12 is a co-factor for conversion of methylmalonyl CoA to succinyl CoA and hence MMA in serum can be elevated in patients who are deficient in B12. However, MMA may be elevated in renal failure by unknown mechanisms. This study assessed the effects of chronic renal failure and transplantation on serum MMA.

An assay for serum MMA was developed using gas chromatography/mass spectrometry with selected ion monitoring, after solid phase extraction and derivatisation with cyclohexanol. MMA was assayed in serum samples collected pre- and 3 months-post transplant from 19 patients who had undergone renal transplantation. MMA was elevated (>466 nmol/L) in 84% of pre-transplant samples (median 610 nmol/L), and fell following transplantation (elevated in 21% of post-transplant samples, median 234 nmol/L, $p<0.005$). However, MMA was not correlated with creatinine when analysed within groups of pre-transplant ($p=0.448$) or post transplant ($p=0.181$) patients. MMA was not significantly related to serum B12 for either pre- or post-transplant group.

MMA was analysed at 0, 3, 6, 12, 24 and 36 months post-transplant in 5 of these patients, therefore reflecting variation in renal function within single patients over time. MMA concentration mirrored creatinine in 4 out of 5 cases.

Elevated serum MMA concentrations are a frequent finding in patients with severe renal impairment and decrease following renal transplantation. However serum MMA may not be directly correlated with creatinine which may make interpretation of MMA and hence B12 status difficult in patients with renal failure.

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The effect of fish oil on the concentration of NT-proBNP in patients treated for atrial fibrillation

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There is an increasing body of evidence to suggest that the consumption of oily fish, or the putative active components therein, omega-3 polyunsaturated fatty acids (PUFA), may have a beneficial effect in reducing cardiovascular disease. The DISAFF (Dietary Intervention Study in Atrial Fibrillation or Flutter) trial has been underway in the Bolton and Wigan area for over 5 years to include over 700 subjects. The rationale of the trial is to investigate the effect of consumption of oily fish on the likelihood that patients with AF remain in sinus rhythm following cardioversion. Participants were randomised either for intervention: given dietary advice of healthy eating plus the addition of 200-400 g oily fish per week, or to a control group who were given advice to follow the healthy diet only.

One offshoot of the study examined the change in a marker for cardiac failure, brain natriuretic peptide (BNP), which is released by heart muscle in response to stretch. Prior to secretion it is cloven into the active BNP and a putatively inactive N-terminal fragment (NT-proBNP), which has a longer half-life.

It was found that there was a negative correlation between the change in erythrocyte omega-3 PUFA content (an index of fish oil consumption) and change in NT-proBNP ($r= -0.44$, $p<0.005$). These results suggest that the beneficial effect of omega-3 PUFA may extend to an improvement in cardiac function, in addition to the antiarrhythmic effects primarily being investigated in the DISAFF trial.

60
Enteral nutrition and elevated whole blood manganese

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Manganese is classed as an essential micronutrient but its biological functions in man remain uncertain. Manganese toxicity is well documented after pathological absorption through the lungs in manganese ore miners. Toxicity has also been described in long-term total parenteral nutrition patients with liver dysfunction. These cases have a Parkinsonian type syndrome and brain MRI scans revealed Mn deposition in the basal ganglia. This report describes a 37-year-old female patient who after seven years on overnight enteral feeding supplementation was noted to have a whole blood manganese concentration = 606 nmol/L (Reference range 70-280 nmol/L). She did not show any clinical evidence of Parkinson's syndrome and a brain MRI scan was normal This patient has a coagulopathy (heterozygous for Factor V Leiden), which had resulted in coeliac axis thrombosis with subsequent gastrectomy, Roux-en-Y oesophago-jejunostomy and liver revascularisation. At initial presentation her liver function tests were deranged with AST = 260 IU/L and ALT = 225 IU/L but over the past two years have been within reference limits. At the time of surgery minimal small bowel was removed and histology did not show any evidence of inflammatory

bowel disease. Due to chronic undernutrition, with a weight at presentation of 36 kg, supplemental enteral feeding was commenced. At present her nutritional regime involves overnight enteral feeds, containing an average of 2.5-5 mg manganese, which is within the average UK dietary intake. The mechanism of manganese absorption is poorly understood but toxicity through oral ingestion has not been reported. The main control of manganese balance is through excretion via the bile. The association between high blood manganese levels and enteral nutrition does not appear to have been previously described.

61
Two cases of copper deficiency in patients receiving home enteral nutrition

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The trace elements selenium, copper, zinc and manganese were measured in the plasma of 37 adult patients receiving long-term home enteral nutrition and 46 control group subjects. No overall significant difference in the trace elements selenium ($p=0.82$), manganese ($p=0.2$), copper ($p=0.7$) was found between the patient and control groups. Plasma zinc concentrations were significantly lower ($p=0.003$) in the patients compared to the control group. Two patients had severely low plasma copper concentrations of 2.4 and 2.5 μmol/L. Further biochemical and clinical examination of these patients ruled out Wilson's disease and acaeruloplasminaemia as a cause of the low plasma copper. Both patients had been fed using a fibre enriched enteral feed, Jevity (Abbott Laboratories) and were receiving at least 33 μmols of copper daily. Three other patients receiving Jevity had plasma copper concentrations within the range of the control group. Neither of the patients with copper deficiency demonstrated the anaemia and neutropenia associated with long term copper deficiency.

Both copper deficient patients were treated with a multi-nutrient solution, Minedex (Seven Seas Health Care Ltd), via their gastrostomy tube at a different time to the administration of their Jevity feed. This supplemental copper (6.3 μmol/day) increased plasma copper in one of the patients to 12.2 μmol/L after 69 days of treatment. The other patient initially showed little change in plasma copper until the Mindex was administered at a different time to his daily drug treatment regime and plasma copper rose to 4.7 μmol/L.

The malabsorption of copper from the Jevity feed in these patients requires further investigation.

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Elevated blood lead levels in renal dialysis patients: a possible association with water lead content

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A single renal dialysis patient showing possible symptoms attributable to lead toxicity, was found to have consistently elevated blood lead levels (187-283 μg/L, n=6). After a pilot study (n=10) which showed elevated blood lead in patients at a single dialysis unit, we decided to screen all patients, at five separate dialysis units (located in two different Water Authority regions), together with those patients receiving home-haemodialysis.

EDTA blood samples were taken from 286 patients in total, from dialysis units A-E and from home-haemodialysis (F). Water samples, both before and after treatment together with dialysis fluid samples were taken from each unit and also from home-haemodialysis locations. All samples were taken within a four-week period and were analysed by graphite-furnace atomic absorption spectrometry.

The mean (SD) blood lead concentrations for units A to F was A: 153(60.9) μg/L, n=98; B: 166.3(41.6) μg/L, n=62; C: 187.8(59.0) μg/L, n=29; D: 162.3(52.7) μg/L, n=57; E: 72.4(32.1) μg/L, n=25 and F: 192.6(88.2) μg/L, n=14. A normal range of <104 μg/L and hazardous range of >601 μg/L is quoted. The blood lead concentrations from unit D were shown to be significantly lower those at any of the other units (students t-testing, $p<0.0001$). Preliminary results of the water and dialysate analysis has shown that despite all fluids having lead concentrations below the Renal Association (RA) limit of 5 μg/L, significant differences between units have been found. It appears that unit D has the lowest concentrations of lead in water of those dialysis units studied.

Elevated blood lead levels have been identified in renal dialysis patients in 4 out of 5 renal dialysis units. From preliminary data, there appears to be a correlation between the blood lead levels found in dialysis patients and the water supply used at individual renal dialysis units.

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Practical considerations for the use of BNP

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Recent studies have shown that B-type natriuretic peptide (BNP) is a good marker for ruling out heart failure. The use of BNP is now recommended by the European Society of Cardiology and is included in the

NICE guidelines. The objective of this study was to evaluate the utility of BNP in a primary care setting. Samples containing EDTA in plastic tubes were taken from 99 patients attending echocardiography appointments. The samples were separated and frozen within 4 hours and assayed on the Bayer Centaur immunoassay analyser. A technical evaluation of the method was performed and BNP results were compared with echocardiography data. The effect of age, gender and treatment were investigated.

The precision at three different concentrations was acceptable (CVs were <7.1%) and the BNP had a minimum recovery of 82% at 24 hours when stored unseparated at room temperature. At a cut-off level of 100 pg/mL sensitivity, specificity and negative predictive value were 47%, 78% and 74% respectively. However, the data showed that both age and treatment had a significant effect on BNP levels. BNP values increased markedly with age and the use of age related cut-off values improved the negative predictive value from 74% to 78%. Patients undergoing treatment for heart failure had lower levels of BNP. Median BNP values for patients with impaired LVF, treated and untreated were 88 and 139 pg/mL respectively ($p<0.05$). The exclusion of patients undergoing treatment improved the negative predictive value further to 89% (using age-related cut-off values). The data showed no difference between male and female patients.

The study showed that age and treatment status are important factors in the interpretation of BNP results. BNP has now been introduced as a routine assay for primary care and its use will be audited.

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The role of B-type natriuretic peptide in the prediction of cardiovascular support and hospital resource utilisation after cardiac surgery

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Cardiac surgery is associated with a significant risk of morbidity including stroke, myocardial infarction and malignant arrhythmia, with a quoted incidence of up to 22%. These have a significant impact on patients' quality of life and the cost-effectiveness of surgery. We have assessed the significance of pre- and post-operative measurements of BNP in predicting the level of post-operative cardiac support and overall hospital utilisation of resources.

Fifty consecutive patients undergoing elective cardiac surgery were selected for this study. EDTA blood samples were taken pre-operatively and post-operatively at 6, 24 and 72 hours. Samples were analysed for BNP using the Bayer Advia Centaur automated analyser. Clinical data was retrospectively collected from patient case notes and included level of post-operative inotropic support, intensive care unit (ICU) length of stay (LOS) and overall total hospital LOS.

Pre-operative levels of BNP (pg/mL) were found to be higher in patients requiring significant inotropic support (median 150 versus 41, $p=0.01$), increased ICU LOS (median 188 versus 53, $p=0.001$) and increased overall hospital LOS (median 92 versus 38, $p=0.06$). By 24 hours post-operatively this difference had increased such that BNP levels were higher in patients requiring significant inotropic support (median 522 versus 282, $p=0.001$), increased ICU LOS (median 1164 versus 310, $p=0.001$) and increased overall hospital LOS (median 464 versus 315, $p=0.03$). Similar trends were observed for BNP levels measured at 6 and 72 hours post-operatively.

This pilot study has demonstrated that BNP levels measured pre and post-operatively may be a useful indicator of subsequent post-operative requirements and may allow hospital resource utilisation to be better planned for.

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Chronic stable ischaemia protects against myocyte damage during beating heart surgery

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Beating heart coronary artery surgery requires a shorter period of coronary artery occlusion than conventional bypass surgery. We investigated myocardial damage caused by this procedure by studying troponin T release.

Thirty-three patients with stable angina and normal left ventricular ejection fraction were studied. Five patients had single vessel disease, 7 had triple vessel disease, and 21 had 2-vessel disease. Of the 21 with 2-vessel disease, 10 were randomised to undergo an ischaemic preconditioning protocol. Intraoperative transoesophageal echocardiography with left ventricular pressure measurement was used to calculate left ventricular regional work and power, as measures of regional wall dynamics. Cycle efficiency (CE), a sensitive marker of regional myocardial ischaemia, was also determined. Myocyte injury was assessed from venous troponin T concentrations measured before, and at 24, 48, and 72 hours post surgery.

Sixteen patients demonstrated regional ischaemia (CE<70%), and 17 did not. The ischaemic patients had lower resting values for regional peak power and work, which increased significantly within 30 minutes of

revascularisation after surgery. Venous troponin T concentration was significantly lower at 48 and 72 hours after surgery in those with ischaemia (median [interquartile range] 0.13 [0.08-0.20] vs. 0.21 [0.13-0.69] at 48 hours, and 0.10 [0.08-0.19] vs. 0.26 [0.12-0.51] at 72 hours, $p<0.05$). Multiple linear regression demonstrated longer ischaemic times and increasing CE values as independent determinants of troponin T release ($p<0.05$). In patients undergoing the ischaemic preconditioning protocol, troponin T release was significantly higher at 72 hours than in the non-preconditioned group (0.24 [0.11-0.36] vs. 0.11 [0.09-0.16, $p<0.05$). No differences were seen in regional wall dynamics between these groups.

In patients with chronic coronary artery disease, stable preoperative ischaemia may represent a naturally occurring form of myocardial protection, whose presence reduces myocardial injury after beating heart surgery. This protection is different in nature from classical ischaemic preconditioning.

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The significance of post-operative serial troponin I levels in 1413 patients undergoing cardiac surgery

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Cardiac surgery for coronary artery bypass grafting (CABG) or for repair/replacement of damaged valves is associated with significant morbidity and mortality that impacts heavily on hospital resources. Interpretation of cardiac troponin levels measured post-operatively can be difficult owing to the release of this marker as a result of the procedure itself. We have assessed the value of such a marker in patients undergoing cardiac surgery, in terms of its diagnostic and prognostic value.

1413 consecutive patients undergoing cardiac surgery (CABG or valve surgery) were selected for inclusion in this study. Blood samples were taken at 0, 24, 48 and 72 hours after completion of surgery and analysed for troponin I (Bayer Advia Centaur assay). Pre and post-operative clinical data were extracted from the patient case notes and hospital information systems.

Troponin levels peaked at 24 hours, with progressively higher levels found as the number of vessels grafted increased. Valvular heart surgery patients demonstrated the highest troponin levels at all time points. Significantly higher levels of troponin at 0 and 24 hours were found in those patients who died within 30 days, required intensive inotropic support, required intra-arterial balloon pump assistance or who had a prolonged intensive care length of stay compared to those who did not. Furthermore, troponin at these timepoints was able to significantly predict these endpoints within a logistic regression model adjusting for sex, age, diabetes, smoking, hypertension, hypercholesterolaemia, and previous myocardial infarction.

Post-operative measurement of troponin in patients undergoing cardiac surgery serves as a marker of myocardial necrosis and provides a useful risk prediction parameter that may also aid resource allocation decision making.

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Perioperative cardiac marker concentrations as predictors of long-term postoperative adverse outcomes in high-risk surgical patients

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Perioperative measurement of the serum concentrations of cardiac troponins T and I (cTnT and cTnI) and of creatine kinase MB (CKMB) can predict major cardiovascular outcomes (including cardiac death) at 3 months post-surgery. In this study, we have followed the postoperative course of 157 patients undergoing major vascular or major orthopaedic joint arthroplasty to one year, using a patient questionnaire, general practitioner follow-up, and case notes review. Cardiac troponins and CKMB were measured before surgery, and on days 1, 2, and 3 post-surgery. The predictive ability of each marker for major cardiovascular outcomes was studied.

Increased post-operative marker concentrations were defined as values greater than the upper reference limit. Increases in cTnI and cTnT concentrations, as well as a single elevated CKMB concentration, and 2 successively elevated CKMB concentrations (CKMBx2), were observed in 12, 13, 33, and 15 patients respectively. Thirty-nine major cardiac outcomes (cardiac death; myocardial ischaemia; congestive cardiac failure; unstable angina; cerebrovascular accident; major arrhythmias needing active treatment) were recorded. Seven of the 12 patients with increased cTnI suffered major cardiovascular complications. Similarly, 5 of 13 with increased cTnT, 10 of 33 with increased CKMB, and 8 out of 15 with increased CKMBx2 suffered major cardiovascular complications.

All 3 markers were positive markers for early cardiac death occurring within 1 month of surgery. There was no significant association between increases in any single marker and cardiac death as a late outcome. However, increases in cTnI and both CKMB and CKMBx2 were associated with an increased incidence of major cardiac outcomes (including cardiac death) to one year [Odds ratio (95% CI): 4.19 (1.65-14.87); 3.97 (1.65-9.44); and 5.19 (1.60-16.22) respectively].

68
Elevated troponin I levels in cardiomyopathy

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Cardiomyopathy is a disease of the myocardium that may eventually lead to cardiac failure. Currently, there are over 200,000 people in the UK who have cardiomyopathy. Troponin I (cTnI) has been used in the diagnosis of acute myocardial infarction (AMI) and in risk stratification of patients with acute coronary syndromes (ACS) particularly as a means of managing patients in the A/E department. However, there is now growing evidence that cTnI may be raised in diseases other than ACS.

The aim was to assess the specificity of cTnI to myocardial injury by measuring its levels in patients with cardiomyopathy who have non-ischaemic coronary vessels. Patient: TP is a 59-year-old Irish male who has had nine admissions since July 2000 for cTnI positive chest pain. He has dilated cardiomyopathy thought to be secondary to chronic alcohol abuse, chronic atrial fibrillation, ventricular tachycardia, hypertension, and some degree of cardiac failure. Since July 2000 he has been admitted nine times. He has had two coronary angiograms that have demonstrated unobstructed coronary vessels.

The cTnI results were confirmed using two established methods, the DPC and the Dade Behring sensitivity of which was 1.0 and 0.03 g/L respectively. TP had an elevated cTnI level of 1.7 using the DPC and 0.28 mg/L Dade Behring with no evidence of ischaemia.

To date we have shown in one patient, with normal coronary vessels as assessed by angiography, that a cTnI level was significantly increased. This increase may be due to extensive remodelling of the myocardium. cTnI is increased in such diverse conditions as rheumatoid arthritis and pre-eclampsia. This observation, if confirmed will have an impact on the management of patients with acute cardiac pain in the A/E department.

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Troponin-T to Troponin-I : a cardiac marker service improvement

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The value of the troponins in the investigation of possible acute coronary syndrome has been firmly established in extensive clinical trials and their use mandated in European and US Cardiology Guidelines. Troponin-T (Tn-T) has been the single most widely applied of these markers and has been available for several years in our laboratories on a twice daily basis, analysed on a Roche Elecsys 1010.

In response to clinical user demand for a more rapid and frequent service, the opportunity to consolidate analytical platforms by adopting the Bayer Centaur Troponin-I (Tn-I) method was investigated.

In a pilot study over three months Tn-I was made available in addition to Tn-T on a limited basis. Tn-T assay frequency remained unchanged with Tn-I provided on a 24/7 basis and a target result availability of 1hr. Of 1683 requests 453 (27%) were for the marker pair. Haemolysis (Hb >0.1 g/L) precluded Tn-T analysis in 49 cases. For 402 data pairs, cutoffs of >0.05 μg/L (Tn-T) and >0.10 μg/L (Tn-I) showed 307 negative and 73 positive for both with 17 (4%) (only Tn-I positive) and 5 (1%) (only Tn-I negative) discordances. Clinical notes for 10 (8 and 2) of these patients were available for review.

In neither of the negative discordances was there other evidence to support acute myocardial injury while in all positive discordances indicators of acute coronary syndrome were noted on review. Two of the largest Tn-I/T differences had subsequent paired tests with both markers confirming myocardial injury. The Tn-I result turnaround target was achieved in 66% of cases; only 2% of Tn-T results were available in that time.

We conclude that Tn-I shows equal negative predictive value to Tn-T, may offer increased sensitivity in detecting ischaemic injury and will, in our laboratories, through continuous rapid availability, enable service provision of a quality better suited to changing clinical needs.

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Serum cardiac troponin I in feline hypertrophic cardiomyopathy

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Ischaemic heart disease is rarely seen in animals, thus for veterinary clinicians the role of cardiac troponin measurement has largely been limited to determining the presence of any cardiac trauma, say following a road traffic accident. However, felines do suffer primary myocardial damage in the form of hypertrophic, usually obstructive, cardiomyopathy, and inherited forms have been demonstrated. These are more prevalent in some breeds than others, e.g. the Maine Coon, which can have obvious consequences for breeders.

Typical morphological changes in hypertrophic cardiomyopathy include myocyte hypertrophy and disarray, with surrounding areas of increased loose connective tissue. Raised cTnI has been shown to be both a sensitive and specific marker of cardiac myocyte injury. Once the molecular structure of cTnI had been demonstrated to be highly conserved across species, we fully validated DPC's Immulite cTnI assay, originally marketed for use in

humans, for cats, dogs and horses.

The aim of this study was to compare serum cTnI levels between 16 cats diagnosed with hypertrophic cardiomyopathy, on echocardiographic evidence, with 18 control cats. The DPC assay comprises an immunometric assay, using a monoclonal capture antibody with quantification by chemiluminescence.

The results demonstrated that cats with hypertrophic cardiomyopathy show significantly raised concentrations of serum cTnI (range 0.2-4.1 ng/mL) compared with control cats (range <0.2 ng/mL). Dilutional parallelism and assay precision were good (intra- and inter-assay variability at 5 levels ranged between 1.8-5.7% and 2.9-7.8% respectively).

In conclusion, DPC's Immulite cTnI assay appears to offer a useful tool in the differentiation between cats with cardiomyopathy and those without.

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Nitric oxide metabolites in symptomatic electrocardiographic ischaemia

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The aim of this study was to measure plasma nitrite and nitrate during symptomatic electrocardiographic ischaemia

Nitric oxide (NO) plays a pivotal role in the pathophysiology of coronary heart disease (CHD). Low plasma concentrations of NO metabolites (nitrite and nitrate), the stable oxidation products of NO have been reported in patients with CHD but this is controversial. Plasma nitrite and nitrate concentrations during symptomatic myocardial ischaemia and in response to exercise in subjects with CHD have not been studied. We therefore measured plasma nitrite and nitrate concentrations in subjects before and after an exercise tolerance test (ETT).

Plasma nitrite and nitrate concentrations were measured before and after an ETT in 24 subjects with symptomatic exercise-induced myocardial ischaemia (positive ETT) and in 27 subjects without exercise-induced myocardial ischaemia (negative ETT).

Plasma nitrate concentrations were higher ($p<0.002$) before and after the ETT in subjects with a positive ETT (31.51±21.80 μmol/L and 30.86±21.42 μmol/L respectively) than in the subjects with a negative ETT (14.75±6.71 μmol/L and 15.64±6.50 μmol/L respectively). Plasma nitrite concentrations before and after the ETT were similar in both groups. Within each group, plasma nitrite and nitrate concentrations were not altered by exercise.

Subjects with exercise-induced myocardial ischaemia have higher plasma nitrate concentration than subjects without exercise-induced myocardial ischaemia. This is consistent with either a compensatory or an inflammatory response of the vascular endothelium to endothelial damage. Symptomatic exercise-induced ischaemia is not associated with altered plasma NO metabolite concentrations.

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Plasma nitrotyrosine and reversible myocardial ischaemia

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The aim of this study was to measure plasma nitrotyrosine concentration in patients with reversible symptomatic myocardial ischaemia

Nitric oxide (NO) is an important cardioprotective molecule synthesised in the vascular endothelium from nitric oxide synthase. However, NO may have detrimental effects under oxidative stress conditions such as in myocardial ischaemia and reperfusion, where in the vascular endothelium and cardiac myocytes it combines with superoxide to form a highly reactive oxidant species, peroxynitrite. Peroxynitrite is capable of inducing tissue injury and can aggravate myocardial damage. Peroxynitrite nitrates plasma free and protein bound tyrosine residues to yield nitrotyrosine, which has been used as a marker of peroxynitrite mediated tissue damage. Plasma nitrotyrosine, however, has not been previously measured during reversible symptomatic myocardial ischaemia (angina). We, therefore, compared plasma free nitrotyrosine concentrations before and after an exercise tolerance test (ETT) used to diagnose myocardial ischaemia.

Plasma free nitrotyrosine concentrations was measured before and after an ETT in 24 subjects with symptomatic exercise-induced myocardial ischaemia (positive ETT) and in 27 subjects without exercise-induced myocardial ischaemia (negative ETT)

All patients with a positive ETT developed chest pain during exercise. Plasma nitrotyrosine concentrations before and after the ETT were undetectable in all subjects.

We report undetectable plasma nitrotyrosine in patients with symptomatic reversible myocardial ischaemia and their controls. Several studies have reported elevated plasma nitrotyrosine in patients with myocardial ischaemic-reperfusion injury but, undetectable plasma nitrotyrosine in healthy controls. Although increased plasma nitrotyrosine as marker of peroxynitrite formation is a feature of severe endothelial insult, our results suggest that the short lived and reversible myocardial ischaemia (angina) as induced by exercise is not sufficient to generate an oxidative stress

milieu and micromolar quantities of nitric oxide to overcome the scavenging role of superoxide dismutase and produce measurable peroxynitrite levels.

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Relationship between serum inflammatory markers and coronary risk score in Saudi males

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Coronary heart disease (CHD) is a chronic inflammatory disease, and an association between serum inflammatory markers and coronary risk that is independent of traditional risk factors has been demonstrated previously. The Framingham risk score (FRS) is used to assess CHD risk, but may not be accurate in non-Caucasian populations, however it has been shown to correlate with serum C-reactive protein (CRP) levels. We aimed to investigate the relationship between FRS and other components of CHD risk and several serum inflammatory markers in a Saudi male population without CHD.

Subjects without CHD (n=138) were recruited from the King Abdul Aziz University. Subjects with renal or hepatic disease were excluded, as were individuals on antioxidant treatment, statins or aspirin. Fasting blood samples were taken for assessment of lipid profile, high sensitivity(hs)-CRP, soluble intercellular adhesion molecule-1 (sICAM-1) and caeruloplasmin (Cp). The former analytes were measured by ELISA, the latter by ferrioxidase activity. Body mass index (BMI), physical activity, and FRS (age, total cholesterol, HDL, blood pressure, smoking) were assessed for each subject. Univariate statistical analysis was undertaken between inflammatory markers and each of these variables. Median serum sICAM-1, hsCRP and Cp were 246 ng/mL, 7.01 mg/L and 209.4 U/L respectively. There was an inverse relationship between sICAM-1 with age and FRS ($r=-0.182$ and 0.175 respectively, $p<0.05$ for both). CRP was positively associated with BMI ($p=0.002$). CRP was weakly correlated with Cp ($r=0.202$, $p<0.05$) but not with sICAM-1. The relevance of the FRS to non Caucasian populations has been challenged previously. It appears that in the Saudi population the relationship between FRS and serum inflammatory markers is not as strong as in a Caucasian population.

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Impact of ESC/ACC Consensus Document on clinical biochemistry departments in Scotland

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The Joint European Society of Cardiology/American College of Cardiology (ESC/ACC) Committee Consensus Document redefined the diagnosis of myocardial infarction and placed greater importance on the use of cardiac troponin (cTn) measurements in making the diagnosis and influencing treatment of patients presenting with chest pain.

To evaluate the impact of these changes on laboratories the Scottish Audit Group issued a questionnaire to twenty-three biochemistry laboratories throughout Scotland. The aim was to evaluate the consistency of the service offered throughout the Scottish region, and to determine how successful laboratories had been in implementing the new ESC/ACC cut-off values for cTn and assessing the precision of their methods at these cut-off values.

The questionnaire was issued in December 2002, 16 laboratories responded (70%) with 13 labs providing a service for the measurement of cTn.

The level of service offered by laboratories was consistent and the majority recommend testing on one sample taken >12 hours after the onset of symptoms with the measurement of CK activity being the most common routinely offered additional cardiac marker.

Many laboratories admitted to being unable to state the precision of their method (either via the manufacturer or internal assessment) at low concentrations of cTn. This was partly explained by the lack of commercially available quality control material of suitably low concentration.

The ESC/ACC redefinition of myocardial infarction and the change in the use of cTn results has exposed both diagnostic companies and clinical laboratories to a clinical demand for increased method sensitivity that some current methods were never designed to meet. However, if results from existing methods are to be used in the risk stratification of patients, it is clear that some laboratories will have to determine for themselves the performance of their methods at low concentrations.

75
An audit of routine homocysteine measurement in patients attending a lipid clinic

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Hyperhomocystinaemia is an established cardiovascular risk factor. It is a modifiable risk factor and intervention with folate supplementation return homocysteine levels to normal within four to six weeks. The aim of this audit was to establish the benefit of routine base-line homocysteine measurements in patients attending our Lipid clinic. Homocysteine was measured in 86 consecutive patients using a dedicated HPLC analyser; (Drew Scientific Ltd, DS30Hcy, reverse phase HPLC with

fluorescence detection; reference range 5.4-15.2 μmol/L). Results showed a bimodal distribution. In Group A, homocysteine results were within the normal reference range with a mean homocysteine result of 9.7 μmol/L (n=73; range= 3.3-14.9 μmol/L). Group B patients had a mean homocysteine result of 22.8 μmol/L (n=13; range=15.4-42.1 μmol/L). Patients in Group B also had a higher blood pressure and significant history of thrombo-embolic events e.g. previous/recurrent strokes and transient ischaemic attacks. Homocysteine may be worth measuring as a modifiable risk factor in our Lipid clinic patients with thrombo-embolic risk factors.

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Ten-year absolute coronary heart disease risk in patients with diabetes: predicted by Framingham Risk Function and UKPDS Risk Engine

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The Framingham Risk Function (FRF) is considered as the gold standard for estimation of 10-year absolute coronary heart disease (CHD) risk. Concerns exist about its accuracy in diabetic populations since the proportion of subjects with diabetes in the Framingham Heart Study was 6%. This study compares CHD risk scores predicted by the UKPDS Risk Engine (UKRE) and FRF in a secondary referral diabetes clinic.

As part of the Alphabet POEM Audit, CHD risk scores using the FRF and UKRE were used as a novel audit tool to assess change in clinical care. Diabetes and cardiovascular parameters were analysed on 400 consecutive patients; 85 were excluded from risk estimation as they had CHD. Risk scores were estimated at referral (T0) and follow-up (Tfu). Statistical analyses were performed using the SPSS package for Windows.

Mean (1SD) CHD risk scores were 24.8(15.6)% from UKRE and 16.8(8.7)% from FRF. A linear relationship existed between the two calculators ($r=0.8$; $n=308$; $p\leq0.0001$). When the CHD risk data was categorised into treatment ranges of ≤15% and >15% as suggested in NICE guidelines, the FRF classified 158 (51.3%) of patients below the 15% treatment cut-off. Of these 158 patients, the UKRE would classify 56 (35.4%) in the >15% treatment cut-off. Conversely, in those which the FRF classified as >15% (48.7%) the UKRE would not treat 10 (5.6%).

UKRE calculations of CHD risk are higher than for the FHF method. These results support the argument that the FRF underestimates CHD risk relative to the UKRE in patients with diabetes.

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Healthier in Yorkshire? the effect of analytical bias on estimates of cardiovascular risk

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In June 2002, the Department of Clinical Chemistry took delivery of Beckmann LX20 analysers as the main general biochemistry analytical platform. Over subsequent months, a negative bias for total cholesterol and positive bias for HDL cholesterol was noted on EQA returns. Despite steps to ensure the assay was working to the manufacturer's specifications, this bias has persisted. Current guidelines recommend that cardiovascular risk (a risk of >30% or >15% in diabetics) is used to guide lipid lowering treatment for patients. This study examines factors associated with the bias and the potential effect on estimates of cardiovascular risk.

Twelve returns for the UK NEQAS scheme for the period October 2002 to November 2003 were examined. Cardiovascular risk was estimated using the Framingham equation for 3 hypothetical patients with low, moderate and high risk factors. Excel and SPSS were used for the statistical analysis.

The mean bias over this period was 0.3 mmol/L. The ALTM was used as a surrogate for the target value (previous returns had shown good agreement between the ALTM and the value for samples returned by a reference laboratory). The negative bias showed a strong dependence on triglyceride concentration ($r=0.86$), the difference between the ALTM and the value returned by this laboratory increasing by 0.16 mmol/L for every mmol/L increase in triglycerides. Few samples had high triglyceride concentrations but in those where it was greater than 2 mmol/L, cholesterol bias ranged from 0.2 to 0.9 mmol/L. Cardiovascular risk showed a corresponding decrease of up to 5% when using lipid values in the equation from our laboratory compared with using the target values.

The Beckmann cholesterol method appears to have a systematic negative bias which is highly dependent on triglyceride concentrations. In patients at risk of cardiovascular disease, especially those with the metabolic syndrome who tend to have higher triglyceride concentrations, this bias may result in significantly lower estimates of cardiovascular risk which might lead to delays in appropriate treatment with lipid lowering therapy.

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Validation of a modified Friedewald equation incorporating lipoprotein (a): the case for an alternative correction or for direct LDL assay?

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Our aim was to validate a correction factor that has been proposed for the Friedewald equation to make allowance

for the presence of lipoprotein (a) [Lp(a)]. Low-density lipoprotein cholesterol (LDL-C) estimated by the Friedewald equation may be significantly over-estimated in the presence of elevated Lp(a). In order to make allowance for Lp(a), a correction to the equation by subtracting 0.3 multiplied by Lp(a) mass has been suggested, though has not been independently validated. We therefore collected fasting blood samples from 209 consecutive cases attending the Lipid Clinic and measured lipids, including LDL-C by direct homogenous assay (Roche Diagnostics) on the Abbott Aeroset analyser as a reference method and Lp(a) by immuno-nephelometry on the Dade Behring BN2 analyser. LDL-C was also calculated with the Friedewald equation with and without the Lp(a) correction and excluding cases with fasting triglycerides >4.5 mmol/L, where the equation is invalid. For LDL-C (derived by the uncorrected equation) versus direct LDL-C, the mean difference for all cases was 0.29 (95%CI 0.25-0.33) mmol/L. The difference was 0.00 (95%CI -0.05-0.06) when using the correction for Lp(a) to the Friedewald equation. Mean difference for cases with Lp(a) in the range 200-500 mg/L (n=48) was 0.00 (95%CI -0.10-0.09) mmol/L using corrected LDL-C and for cases with Lp(a) >500 mg/L (n=60) was -0.36 (95%CI -0.47 - -0.25). The Lp(a) correction to derived LDL-C is therefore valid for the direct LDL-C and Lp(a) methods used in the present study, except in cases with Lp(a)>500mg/L, where there is overcompensation, possibly reflecting that higher mass Lp(a) phenotypes have a lower cholesterol content. There is a case for a more robust correction factor or for direct LDL-C assay. These findings have implications for epidemiological studies, interpretation of clinical trials and patient management.

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A textbook case of Type III hyperlipoproteinaemia: except with a Fredrickson type IIb phenotype

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The patient presented to the lipid clinic with palmar crease xanthomata and hyperlipidaemia. Apo E genotyping confirmed that she was homozygous for Apo E2 and she was diagnosed as having type III (remnant) hyperlipoproteinaemia. Lipid electrophoresis performed on a serum sample from her first clinic visit showed a type IIb pattern, however and this was confirmed at a subsequent clinic visit. The palmar crease xanthomata have responded to treatment with fenofibrate.

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Severe hypoalphalipoproteinaemia during treatment with rosiglitazone

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Thiazolidinedione drugs are in widespread use for the treatment of type 2 diabetes. In addition to improving insulin sensitivity they generally result in a modest elevation of plasma HDL cholesterol. We report three patients, all of whom had pre-existing diabetic dyslipidaemia, who showed a profound reduction in plasma HDL cholesterol and ApoA-I levels soon after the initiation of rosiglitazone therapy. In all three patients HDL cholesterol levels returned to normal following drug withdrawal. The fact that this phenomenon was not seen in over 1,400 patients studied in clinical trials indicates that it is likely to be rare and idiosyncratic. However, until the frequency of this adverse reaction is clearer it would seem advisable to measure plasma HDL cholesterol before and after commencement of thiazolidinedione therapy.

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A comparison of three homogeneous methods for measuring high-density lipoprotein cholesterol with a precipitation method in diabetic and non-diabetic subjects

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The homogeneous assays for measuring HDL-cholesterol (HDL-C) have replaced the conventional precipitation methods in most laboratories. The accuracy of the homogeneous methods has been questioned, specially in liver cirrhosis, a condition associated with significant alterations in lipoprotein structure and composition. Diabetes mellitus is a syndrome associated with significant disturbances of lipoprotein metabolism. The aim of this study is to compare the performance of three homogeneous assays with a precipitation method in samples from patients with type 1 or type 2 diabetes and non-diabetic control subjects. We have also investigated the interference by triglycerides, bilirubin and haemoglobin in the homogeneous assays.

Three homogenous methods for the automated measurement of HDL-C were studied and compared with a precipitation method in patients with type 1 or type 2 diabetes and non-diabetic controls (n=30 in each group). The precipitation method used was the phosphotungstic/$MgCl^2$ method from Boehringer.

All three methods met the precision criteria of the National Cholesterol Education Program (NCEP). Triglycerides did not cause significant interference up to 10 mmol/L in the immuno-inhibition method (WAKO)

and up to 40 mmol/L in the RANDOX and ROCHE methods. Haemoglobin, up to a concentration of 5 g/L, had only negligible effects on the performance of all three homogeneous methods. Bilirubin caused an increasing positive bias in all methods above a concentration of 50 mmol/L. In comparison with the precipitation method, the new homogeneous methods agreed for type 1 diabetic patients but showed a positive bias for the control subjects and patients with type 2 diabetes.

The bias of HDL-cholesterol levels in type 2 diabetes may be sufficient to affect the calculation of cardiovascular risk, and may therefore influence the decision to prescribe lipid lowering medication.

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Development of a direct high density lipoprotein cholesterol VITROS chemistry products slide for use with VITROS chemistry systems

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We are developing a rapid, fully automated thin film method for high density lipoprotein cholesterol (HDLC) in human serum and plasma for use with VITROS chemistry systems. No sample pre-treatment is necessary. In the spreading layer, non-high density lipoproteins are precipitated in the presence of phosphotungstic acid (PTA) and magnesium chloride ($MgCl_2$). A surfactant in the spreading layer aids in the selective dissociation of the cholesterol and cholesterol esters from the HDL lipoprotein complexes present in the sample. Hydrolysis of the HDL derived cholesterol ester to cholesterol is catalyzed by a selective cholesterol ester hydrolase. Free cholesterol is then oxidized in the presence of cholesterol oxidase to form cholestenone and hydrogen peroxide. Finally, hydrogen peroxide oxidizes a leuco dye in the presence of peroxidase to generate a colored dye. The density of dye is proportional to the HDL cholesterol concentration present in the sample and is measured by reflectance spectrophotometry.

Excellent agreement between the VITROS direct HDL slide assay and a comparative method (dextran sulfate/enzymatic method with traceability to the CDC Designated Comparison Method) was obtained. A panel of 70 human serum samples with HDL concentrations between 0.54 mmol/L and 2.40 mmol/L were evaluated, and the correlation results obtained were: slope = 1.04, intercept = -0.03 mmol/L, Syx = 0.03 mmol/L, and r=0.996. The accuracy of the direct HDLC slide was verified using Pacific Biometrics HDL-Cholesterol Verification Specimens with traceability to the CDC Designated Comparison Method. The results for the 5 specimens from Lot 1002 HDLA - 1002 HDLE were: 0.54 mmol/L, 1.04 mmol/L, 1.55 mmol/L, 2.06 mmol/L, and 2.55 mmol/L with reference values of 0.54 mmol/L, 1.06 mmol/L, 1.60 mmol/L, 2.12 mmol/L, and 2.64 mmol/L, respectively. The VITROS direct HDL slide also showed good 10-rep within run precision, with CVs ranging from 0.68% to 2.11%. No significant interference was detected from bilirubin up to 342 mmol/L, from haemoglobin up to 4.0 g/L, and from triglycerides up to 6.77 mmol/L.

In conclusion, the VITROS direct HDL slide assay combines very good analytical performance with the advantages of a rapid, automated, random access test and no sample pre-treatment.

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Evaluation of Freelite assays (The Binding Site Ltd) in the assessment of multiple myeloma

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Urinary free light chain measurement by electrophoresis with scanning has been used for many years to aid the diagnosis and monitoring of Bence Jones myeloma. Over the last 25 years, several immunochemical assays for free light chains have been reported but until recently the production of high affinity antibodies, which are sufficiently specific for free light chains (rather than light chains bound to heavy chains) and suitable for simple, automated assays, has remained elusive.

In this study, new latex-enhanced immunoturbidimetric assays (The Binding Site Ltd) for kappa and lambda free light chains have been adapted for use on the Bayer ADVIA® 1650 and then analytically and clinically evaluated. Overall, these assays showed good analytical performance, especially in terms of precision (interassay CVs of $\leq$5% across the range of clinical interest), sensitivity (functional sensitivity of 3.0 mg/L for kappa and 3.3 mg/L for lambda) and linearity (for both polyclonal and monoclonal samples).

The utility of these assays, in the monitoring of myeloma, has been investigated by analysis of patient samples from the myeloma clinic at Christie hospital. It was concluded, from case studies of Bence Jones myeloma patients (samples collected over 6 months), that determination of free light chains offers a feasible alternative to monitoring of light chain excretion by electrophoresis with densitometric scanning. It was hypothesised that analysis of free light chains may allow earlier assessment of treatment response in intact immunoglobulin-producing myeloma, due to the shorter half-life of free light chains compared to intact immunoglobulins. Data from intact myeloma patient case studies goes some way to supporting this notion,

although further work is required.

In order to maximise the potential of these new and promising assays, a change in clinical practice is required, as well as the development of guidelines for their use.

84
Evaluation of a new Nichols Diagnostics reagent for hyperglycosylated human chorionic gonadotropin

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Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone produced by trophoblast cells in pregnancy, gestational trophoblastic disease (GTD) and gestational trophoblastic tumours (GTT). It is measured in all patients following hydatidiform mole to monitor for GTT development. Hyperglycosylated hCG accounts for up to 100% of hCG produced by GTT, but is lower in GTD. We evaluated the Nichols Institute Diagnostics hyperglycosylated hCG assay (marketed as Invasive Trophoblastic Antigen) on the Nichols Advantage® Analyser.

Imprecision was assessed using quality control samples supplied by Nichols at levels of 2.14 ng/mL, 19.6 ng/mL, 190.5 ng/mL (n=14). Coefficients of variation (CV) were 10.1%, 5.2% and 7.0% respectively. A precision profile indicated that 88% of samples greater than 1 ng/mL had a CV of less than 5%, but 90% of samples less than 1 ng/mL had a CV of greater than 5%. No antigen excess was observed on samples with levels of 32500 ng/mL. The limit of detection determined using non-gestational serum was 0.170 ng/mL. To assess stability aliquots of a sample were stored at room temperature, 4°C, -20°C, and -80°C and assayed over three weeks. No results varied from the original value by more than 10%, except storage for three weeks at room temperature, which showed an increase of 20%.

We investigated whether hyperglycosylated hCG would predict the development of GTT in the hydatidiform mole follow-up population. Patients' (n=33) first and second samples following hydatidiform mole were analysed. There was good correlation between the level of total hCG (measured using our in-house radio-immunoassay) and hyperglycosylated hCG (r=0.84). Patients requiring treatment (n=8) tended to have both high hyperglycosylated hCG and high total hCG.

The new Nichols Diagnostics hyperglycosylated hCG reagent shows good precision and stability, with no detectable antigen excess. Its potential use in hydatidiform mole follow-up patients requires further investigation.

85
Is a prostate biopsy necessary in elderly men with a PSA level greater than 20 µg/L?

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Prostate specific antigen (PSA) testing is used in the management of prostate cancer. It is also being used by GP s as a screening tool for prostate cancer to determine whether further investigation or referral is appropriate. The evidence for the use of PSA in this context is still undetermined. The aim of this study is to determine if there is any correlation between an initial PSA level done by a GP and the corresponding prostate biopsy results.

Results were collated from East Kent Pathology Services which serves a population of 603,000 in the South East of England. The trust follows the guidelines for prostate cancer management as set by the Prostate Cancer Risk Management Programme. Results for an initial PSA requested by a GP were collected and were paired with the corresponding Gleason score from the biopsy.

All prostate biopsies with an index PSA of >20 µg/L were collated for the year 2003. The search resulted in 39 unique data pairs in which an initial PSA was requested by a GP which was then consequently followed up with biopsy and had a Gleason Score.

The most commonly reported Gleason score was 3+3. The age range of the men who had a PSA of over 20 µg/L and a Gleason score was from 56 years of age to 83 years old. The PSA values had a range of 22.6 µg/L to 166.8 µg/L.

The value of the PSA did not in itself predict the Gleason Score and there was no correlation between PSA and age, Gleason score and age or PSA and Gleason score.

From the data obtained it can be concluded that older men (>55 years old) who present with a PSA of over 20 µg/L at GP presentation, most probably have a prostatic carcinoma with a Gleason Score of 3+3. This would suggest that a biopsy is an unnecessary procedure if a Gleason Score of 3+3 has no effect on management or prognosis.

86
Serum HER-2/neu concentrations in metastatic melanoma

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The human gene *c-erbB-2* is located on chromosome 17q21 and encodes a 185 kDa receptor (HER-2/*neu*) with tyrosine kinase activity. Over expression of this

HER-2/*neu* receptor is associated with numerous cancers including breast, prostate, lung and gastric. In breast cancer HER-2/*neu* is used as a prognostic marker and over expression is indicative of a worse prognosis. Recently numerous studies have evaluated the status of HER-2/*neu* in malignant melanoma at a tissue level but results to date are inconclusive.

We have measured serum HER-2/*neu* concentrations in twenty-six patients [male = 18; female = 8, median age 54 years (range 36 to 79)] with metastatic melanoma [American Joint Committee on Cancer (AJCC) grades III & IV]. Assays were carried out on samples collected for S100β measurements, an assay routinely used in the management of patients with malignant melanoma. The extracellular domain of HER-2/*neu* was measured using an automated enzyme immunoassay (Immuno 1, Bayer Diagnostics).

Statistical analysis of the data showed a median HER-2/*neu* concentration of 10.27μg/L (range 4.6 to 16.1 μg/L) with all samples but one giving HER-2/*neu* values within the normal limit of 15 μg/L. The abnormal result (16.1 μg/L) was that obtained in a patient with widely disseminated disease whose S100β levels had peaked at 9.10 μg/L (normal ref range <0.2 μg/L). Interestingly serum HER-2/*neu* concentrations seemed to be lower in stage IV melanoma (median 9.8 μg/L) than in stage III (Median 11.2 μg/L, p=0.05).

Increased serum HER2/*neu* concentrations were not found in this group of patients with metastatic malignant melanoma in contrast to previously published tissue data.

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Does published literature influence the appropriate requesting of CA 19-9?

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CA 19-9 came to prominence when antibody studies showed its reactivity with a mucin antigen involved in T cell apoptosis and thus the malignant process. It was demonstrated that elevated levels occur not only in pancreatic adenocarcinoma (for which it has become synonmous) but also in colorectal and gastric carcinomas. Several non-malignant conditions including pancreatitis, hepatocellular jaundice, cirrhosis, acute cholangitis and cystic fibrosis may also give a raised level. It has also been reported that ten percent of the population will not express CA 19-9 even in the presence of these malignancies. Thus the test is not specific or sensitive enough to be used for screening.

Currently there are many recommendations which exist concerning the appropriate use of CA 19-9, yet to-date there are no formal guidelines for its use within East Kent. This study was designed to determine current practice in requesting CA 19-9 in East Kent against current knowledge that it should only be used once a malignancy diagnosis is reached and specifically to monitor treatment response, screen for recurrence and act as a prognostic indicator.

Seventy-one requests were made for CA 19-9 during November 2003 with 89% of forms recovered. Based on the information provided on the request form 81% of the requisitions were inappropriate according to the recommendations, whilst only 19% were acceptable. Further analysis according to specialty revealed that Medicine showed the highest inappropriate requesting (90%). General practice and Surgery faired little better however with 80% and 75% of their requisitions being inappropriate respectively.

This paper has demonstrated that the despite the large amount of information available in the literature the vast majority of CA 19-9 requisition contained inadequate information to justify the test. This highlights the need for the promotion of local guidelines and education in order to improve appropriate requisition rates and appropriate use of resources.

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How can we minimise inappropriate tumour marker requests?

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The increased requesting of tumour markers prompted an audit of actions taken by clinicians on the results of CA125, CEA and CA199 measurements. Between 1996 and 2001 requests increased by 49% (CA125), 155% (CEA) and 577% (CA199). Evidence based guidelines for the use of these markers are available. The audit was carried out on two groups of patients in whom it was unlikely that guidelines had been followed. Cases were identified using the laboratory computer system from requests made in 2001. Group 1 - two or more markers requested on a single sample; Group 2 - single request for CEA with no follow up measurement.

Group 1: There were 2503 tests for these markers in 2001. 256 requests (557 tests) were for at least two markers in the same patient, 33 requests (99 tests) were for all three. As a percentage of total requests for these markers this represented 17% for CEA, 21% for CA125 and 59% for CA199. In 32/33 cases where all three markers were requested, this was to investigate new, non-specific symptoms. Sensitivity and specificity (of at least one elevated marker) were 75% and 65% for any malignancy. All patients went on to have further diagnostic investigations, often initiated before tumour

marker results were available.

Group 2: There were 800 requests for CEA in 2001. Single CEA measurements were made in 229 patients. A random sample of 36 notes were reviewed. 11 patients were known to have metastatic colorectal cancer. A new diagnosis of malignancy was made in six cases - but further definitive investigations were arranged in all six, in two of whom the CEA result was normal. CEA was elevated in 2/19 patients in whom malignancy was not found.

This audit confirms the poor performance of CEA as a diagnostic test in routine practice, and shows that although frequent requests were made for combinations of CA125, CEA and CA199, results rarely contributed to making a diagnosis.

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Tumour marker kinetics: a pragmatic analytical interpretation of rates

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Graphical reporting of tumour marker (TM) levels has shown remarkable linearity in log concentration against time both for disease progression and response to treatment. Interestingly, the positive slopes in disease progression and negative slopes during response appeared to be similar.

The aim was to establish TM half-lives (t 1/2) following surgical removal of the primary (or other forms of treatment), and doubling times (t2) during disease progression for a variety of different tumours.

Linear changes in log (TM) were established over time frames appropriate to the growth rates of the cancers (generally three times the t2 or t1/2). These were then grouped into positive or negative slopes, cancer type and method of treatment. Each group comprised several hundred patients.

Following successful removal of the primary, the t1/2 for small molecules such as hCG and free PSA had a median value of 2.3 days. Larger molecules showed a median t1/2 of 10 days. When treated with chemotherapy or radiotherapy, the t1/2 was a reflection of the growth rate of the primary. The median t2 for most solid tumours appeared to be approximately 30 days; hCG-producing germ-cell and choriocarcinoma as fast as 3 days; aFP-producing germ-cell tumours approximately 6 days, and Ca prostate carcinoma a median t2 of 128 days.

Failure to reach the median t1/2 following surgery generally indicated the presence of metastases or incomplete removal of the primary. Successful chemo- or radiotherapy appeared to invert the positive rate of the advancing disease into the same but negative rate of apoptosing cells - consistent with both processes being a function of the biochemical rate of the cell. Reduction in the negative rate was a sign of relapse, and likewise a reduction in rate was a sign of effective treatment before the markers had begun to show a fall. Equally valuable was the observation that diagnoses could be made on the basis of linearly rising markers. This is particularly important for Ca prostate carcinoma in that it reduces the dependence on defined reference ranges. It can also identify the most rapidly growing tumours, which pose the greatest threat to life.

90
A method for alpha-1-antitrypsin genotyping using denaturing HPLC

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Alpha-1-antitrypsin (AAT) deficiency is associated with cirrhosis in childhood and pulmonary emphysema in adult life. AAT which is also known as alpha proteinase inhibitor (αPI) protects the lung from damage by inhibiting neutrophil elastase which otherwise destroys lung connective tissue.

Over 90 PI variants have been described. Two common deficiency alleles, the S and Z alleles which are single point mutations occur at 7 and 3% respectively in the UK population. The Z variant is of particular importance since it results in very low plasma concentrations (10-20% of the normal M allele) and is associated with intracellular accumulation of protein and consequent hepatocellular damage in addition to emphysema.

In our laboratory, AAT deficiency is investigated by measurement of serum AAT concentration. If a low protein level is found or there is a family history of AAT deficiency, genotyping for the Z and S alleles is performed. Measurement of C-reactive protein is used to exclude a normal AAT concentration being due to an acute phase reaction. Sequencing of the entire gene to detect rare mutations is performed where a low antigen level is found and the Z or S alleles are not detected.

Our current method for AAT genotyping is a restriction enzyme (RE) method using mutagenic primers. This method has the disadvantage of being slow to perform. As an alternative, the use of denaturing HPLC was investigated. PCR products from normal and patient alleles are mixed, denatured and reannealed to form heteroduplexes which are analysed using the Wave-MD apparatus. The assay is quicker to perform and the results matched the RE method in all cases indicating that the denaturing HPLC is a viable alternative for AAT genotyping.

91
An MS-PCR multiplex method for detection of common polymorphisms in thiopurine methyl transferase

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The thiopurine drugs 6-mercaptopurine, azothioprine and thioguanine are used in the treatment of childhood acute lymphoblastic leukaemia, the management of inflammatory bowel disease, autoimmune hepatitis, systemic lupus erythrematosis, rheumatoid arthritis, dermatological conditions and in organ transplantation. Thiopurine drugs are inactive pro-drugs and must be metabolised into thioguanine nucleotides (TGN) to exert efficacy and toxicity. Thiopurine methyl transferase (TPMT) inactivates thiopurine drugs so less toxic TGNs are produced. The TPMT gene is genetically polymorphic. Life-threatening myelosuppression can occur in the 0.3% of Caucasians who are homozygous for deficiency polymorphisms. Heterozygotes (10%) show intermediate sensitivity. In these patients, thiopurine drugs may still be given but at a much lower dose (up to 15x less). Eight TPMT alleles have been identified but the three most common account for around 90% of low TPMT activity. Around 25% of patients without TPMT deficiency also show sensitivity to thiopurine drugs.

TPMT activity may be measured on red blood cells. The disadvantages of phenotypic measurement are: the assay is not widely performed so samples must be sent away, recent blood transfusion give erroneous results and prior administration of the drugs cause induction of TPMT. The major disadvantage of genetic analysis is that rare polymorphisms will not be detected unless time-consuming sequencing of the entire gene is performed. A rare polymorphism could potentially cause fatal myelosuppression. We have developed a method to detect the three common TPMT polymorphisms simultaneously using mutagenically-separated PCR on the ABI 310 DNA analyser. The method has been validated using sequenced controls. Following PCR, each sample takes 30 minutes for analysis allowing for rapid turnaround.

It is unclear at present how the genotyping and phenotypical measurements should best be incorporated in order to provide a clinical service for users of this important class of drugs. This will require formal testing.

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Development of a genotyping service for the identification of butyrylcholinesterase variants

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Bayer Award

The enzyme butyrylcholinesterase (BChE) is essential in terminating the effect of muscle relaxants, such as suxamethonium. Over 20 mutations, resulting in a decrease or loss of enzyme activity within the *BCHE* gene, have been described. Affected individuals suffer from prolonged apnoea when given an otherwise safe dose of a muscle relaxant. The phenotypic assay is limited in that it cannot reliably distinguish between certain mutations, especially where multiple mutations are present.

DNA sequencing of two regions of the *BCHE* gene has been developed to detect the clinically significant K- and atypical variants. The method was then used to genotype 24 patients that had originally had a phenotype assigned based on current methodology. Fourteen of these patients were shown to have more than one mutation present and five of these patients were reclassified as having potential sensitivity as a result. Two patients with queried U/AK or A/K phenotypes were shown to be AK/K genotypes. The AK/K genotype is associated with increased sensitivity to muscle relaxants.

The newly developed genotyping assay will improve the current classification, which is based on phenotypic data alone and will add information about multiple mutations. Interpreting the genetic background in conjunction with measurements of the actual enzyme activity status will enhance the service providing a more detailed, individual diagnosis.

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Reverse-hybridization assay for mutations associated with hereditary sugar intolerance

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A variety of genetically determined enzyme and transporter deficiencies may cause hereditary intolerance to common dietary sugars. Lactose intolerance (adult-type hypolactasia lactase non-persistance) is an extremely frequent autosomal recessive condition causing diarrhea, nausea and flatulence. It is highly associated with two mutations located upstream from the lactase-phlorizin hydrolase (LPH) gene locus. Hereditary fructose intolerance is an autosomal recessive disorder caused by mutations in the aldolase B gene. Affected subjects suffer from severe abdominal pain, vomiting, hypoglycaemia, and unless fructose-containing food is strictly avoided may even die from irreversible damage of the liver and kidney. We have developed a reverse-hybridization assay for the rapid and simultaneous detection of two mutations (-13910 C/T, -22018 G/A) upstream to the LPH gene and four mutations (4E4, A149P, A174D, N334K) in the aldolase B gene. The test is based on multiplex DNA amplification and hybridization to a test strip presenting a parallel array of allele-specific oligonucleotide probes

for each mutation. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and hybridization/detection may be carried out manually or essentially automated using existing instrumentation (e.g. TECAN profiBlot). The test is simple and convenient, requires very small amounts of samples, and can easily be modified to include additional mutations.

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The use of DNA sequencing in the diagnosis and management of metachromatic leucodystrophy

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Metachromatic leucodystrophy (MLD, MIM 250100) is an autosomal recessive demyelinating disease caused by a deficiency of the lysosomal enzyme arylsulphatase A (ARSA; EC 3.1.68.). Mutations within the gene encoding this enzyme (*ARSA*) are usually, but not exclusively, the cause of this deficiency. As most MLD mutations are unique within families, DNA sequencing is usually required for mutation identification. The small size of *ARSA* (less than 2 kB) makes this a plausible diagnostic method, particularly as enzymatic diagnosis of this condition, based on ARSA activity, is complicated by the widespread occurrence of a pseudo-deficiency allele. Individuals homozygous for the pseudo-allele and patients affected by MLD may both exhibit profoundly deficient ARSA activities. As 1% of the Caucasian population is homozygous for the pseudo-allele, the likelihood of finding this in MLD families of this ethnic background is high. Pseudo-allele status is thus essential for interpretation of ARSA enzyme activities. We report here the use of DNA sequencing for mutation detection and have identified 5 previously unknown mutations associated with this condition. We also report the use of DNA sequence information as a method for pseudo-allele detection, identification of carrier status, prenatal diagnosis and monitoring of graft function when bone marrow transplantation has been used as a treatment for this condition.

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The use of denaturing HPLC in the identification of genetic mutations in Menkes disease

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Menkes disease is a lethal X-linked recessive disorder of copper metabolism caused by mutations in the ATP7A gene, usually diagnosed by quantitation of plasma copper and caeruloplasmin. Determination of the genetic mutation is important for reliable carrier detection and antenatal diagnosis. Point mutations are thought to account for around 80% of mutations in this disease. Traditional methods of point mutation detection such as single-strand conformational polymorphism (SSCP) can be time consuming and insensitive, particularly for a gene with such a large coding sequence (150 kb). Currently there is no service available in the UK for the molecular diagnosis of Menkes disease.

Denaturing high performance liquid chromatography (dHPLC) is a sensitive and specific method for the detection of point mutations. This method relies on the tendency of mutated DNA to form heteroduplexes when mixed with wild type DNA. These are detected using their characteristic HPLC elution profile when compared to that of normal control DNA.

dHPLC conditions have been optimised for each of the 23 exons of the ATP7A gene (26 fragments in total) using experimental and computer predicted melt temperatures. The method was then validated using the DNA of five patients and two carriers in whom a mutation of the ATP7A gene had been previously characterised. PCR was used to amplify sections of the ATP7A gene and prior to analysis the PCR products of male patients were combined with that of normal controls to allow heteroduplex formation.

The developed dHPLC method detected mutations in all seven positive control DNA samples analysed. This 100% mutation pick-up rate compares favourably with other methods. dHPLC is suitable for routine use in the molecular diagnosis of Menkes disease, the detection of carriers and has potential for use in prenatal diagnosis.

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Utility of screening for TPMT genotype and phenotype activity in patients commencing on azathioprine therapy

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Azathioprine is an extremely useful drug for the treatment of relapsing inflammatory bowel disease (IBD) and certain dermatological conditions. Administration of the drug results in adverse reactions in 15% of individuals, the most dangerous of which is myelosuppression. This is usually an early event, seen in 3.2% of patients and posing a mortality risk. Deficiency of the hepatic enzyme thiopurine methyltransferase (TPMT) is responsible for a proportion of cases of myelosuppression. Detection of TPMT gene polymorphisms allows identification of

patients who are homozygous (0.3%) or heterozygous (11%) for alleles predicting low TPMT enzyme levels. The three most common inactivating polymorphisms for TPMT are, *3B, *3C and *2. An allele is known as *3A if it carries both the *3B and *3C changes.

The aim of this study was to evaluate the clinical utility of TPMT gene assays compared with TPMT activity measurements in a consecutive series of patients who have used or were due to use azathioprine therapy.

The study subjects were 44 patients with IBD and 19 with skin conditions. Polymorphisms *3B, *3C and *2 were determined. TPMT enzyme activity measurements were carried out and adverse events were noted.

We detected one homozygote for *3A, 3 heterozygotes for *3A and one heterozygote for *3C. This frequency of polymorphisms is in line with previously published work. TPMT enzyme activity was lowest from the homozygote, heterozygotes had intermediate activity and genotypically normal individuals had the highest activities. On the basis of these results, the *3A homozygote did not commence azathioprine therapy. The four heterozygotes were from the IBD group of patients and two of these were no longer on azathioprine. However, other serious adverse events occurred in 8 individuals who were genotypically wild type, with TPMT activity in the normal range.

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Investigating a large family with hereditary coproporphyria

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Hereditary coproporphyria (HCP) is the least common of the autosomal dominant acute hepatic porphyrias and results from mutations in the gene that encodes for the mitochondrial enzyme coproporphyrinogen oxidase (CPO). Biochemical investigations including total faecal, urine and plasma porphyrins, urine PBG, plasma fluorescence emission scanning and faecal copropoprhyrins (measured by HPLC) were performed on 20 members of a large family with an index case of HCP. Blood was also taken for DNA sequencing and 14 members of the family were found to have a mutation (c.119del A) in the CPO gene located within the translated region of exon 1. The most consistent biochemical abnormality in those subjects carrying the mutation was an elevated ratio of faecal Coproporphyrin III: Coproporphyrin I isomer (CIII:CI ratio). This ratio ranged from 3.86 to 35.8 in the family members with the mutation and 0.36 to 1.36 in those with the wildtype. Two of the subjects with a raised CIII:C1 ratio had a total faecal porphyrin excretion within the reference range. Nine of the family members with the mutation had a raised urine porphyrin: creatinine ratio and only 3 had raised concentrations of plasma porphyrins.

Only the index case in this family suffered from severe symptoms of acute abdominal pain associated with HCP. Other family members had much milder symptoms, which could be attributed to the porphyria or were asymptomatic. All subjects were asymptomatic at the time of sample collection and showed no increased excretion of porphobilinogen in the urine.
This work demonstrates the usefulness of faecal CIII:CI ratio as a marker for HCP in this family.

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Identifying the sequence elements important for mitochondrial targeting of coproporphyrinogen oxidase

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Bayer Award

Hereditary coproporphyria (HCP) is an autosomal dominant disorder of haem synthesis due to deficient coproporphyrinogen oxidase (CPO) activity. CPO is the sixth enzyme of the haem biosynthetic pathway and is located within the inter-membrane space of mitochondria where it catalyses the oxidation of coproporphyrinogen III to protoporphyrinogen IX. CPO is nuclear encoded and synthesised on cytosolic ribosomes as a preprotein that contains a presequence of 110 amino acids at the amino terminus. The aim of this study was to identify the sequence elements necessary to target CPO to mitochondria.

We have fused human CPOs containing N-terminal and C-terminal deletions, to the amino terminus of yellow fluorescent protein (YFP) and have used these constructs to investigate the mitochondrial import of CPO in human cells. Constructs were transfected by lipofection into HeLa cells and their cellular location imaged by fluorescence and confocal microscopy. Inspection of the CPO presequence predicts a bipartite structure with dual targeting and sorting information: a matrix-targeting signal consisting of a positively charged region (residues 1-69) followed by an extended hydrophobic sorting region (residues 70-103) that directs the protein to the inter-membrane space. Here we have shown that the residues 1-69 contain all the information necessary to target YFP to the mitochondria. We are currently investigating the role of the hydrophobic region in localising CPO to the inter-membrane space. This and other findings will help identify some of the molecular defects causing hereditary coproporhyria.

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Evaluation of mutation screening as a first line test for the diagnosis of primary hyperoxaluria types 1 and 2

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A definitive diagnosis of primary hyperoxaluria type 1 (PH1) and primary hyperoxaluria type 2 (PH2) requires the measurement of alanine:glyoxylate aminotransferase (AGT) and glyoxylate reductase (GR) activities respectively in a liver biopsy. The logistics of shipping samples to a reference centre on dry ice makes access to the test problematic for smaller centres and for laboratories outside the United Kingdom. The aim of this study was to evaluate the usefulness of a molecular genetic approach for the diagnosis of these autosomal recessive diseases, encoded by the AGXT and GRHPR genes respectively.

The frequencies of three common mutations in the AGXT gene (c.33_34insC, c.508G>A and c.731T>C) and one, c.103delG, in the GRHPR gene were assessed by PCR and mutation analysis of DNA samples from 365 unrelated patients referred for diagnosis of PH1 and/or PH2 by liver enzyme analysis. These mutations account for approximately 43 and 38% of mutant alleles in PH1 and PH2 respectively.

One or more of these mutations was found in 183 (68.8%) biopsy proven cases of PH1 and PH2. 100 (34.8%) patients were homozygous or compound heterozygous for mutations and thus a molecular diagnosis was possible. The sensitivity of mutation analysis was 62.3% for PH1 and 33.3% for PH2. The predictive value of a negative test was 62.0% for PH1 but only 2.2% for PH2 reflecting the much lower frequency of PH2 patients in the population studied.

We conclude that limited mutation analysis can provide a useful first line test for PH1 and PH2 in patients in whom primary hyperoxaluria is suspected and in whom secondary causes have been excluded. Those patients in whom a single or no mutation is found can be selectively targeted for liver biopsy.

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APOE polymorphism and susceptibility to multiple sclerosis in a Gulf Arab population

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APOE polymorphism is believed important in conferring susceptibility to some neurodegenerative disorders including multiple sclerosis (MS). It is unclear if this observation holds in all populations.

The aim of the study was to compare APOE allele frequencies between patients with MS and healthy controls in a Gulf Arab population, and, in the patients with MS, to relate these frequencies with clinical features of the disease.

Two groups of subjects were studied: (i) 39 (17M, 22F) patients with clinical MS; (ii) 106 apparently healthy Kuwaiti control subjects. The MS patients had detailed clinical and laboratory evaluation. APOE genotypes were determined in all subjects by PCR methods. Differences in allele frequencies and associations of specific alleles with clinical features were assessed.

There were no significant differences in allele frequencies between patients and controls, although there was a trend towards lower APOE2 frequency in the patients (p 0.09). There was a significant association of the APOE4 allele with female gender in the patients ($p \leq 0.05$), and a trend ($p \geq 0.10$) towards an association of the E4 allele with severe disability.

In Kuwaiti Gulf Arabs, a population with low MS prevalence, no significant associations between APOE genetic polymorphism and susceptibility to MS could be established but there was a trend towards a lower APOE2 frequency with MS, and towards increased frequency of APOE4 in female patients and with severe disease.

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Rationalisation of laboratory test ordering in primary care: The Diagnostic Request Advisory Model (DRAM) Study

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Laboratory services have a central role in supporting the screening, diagnosis and management of patients and represent significant expenditure of which around 25% can be attributed to general practice use. Some testing however can be regarded as inappropriate, which represents a substantial opportunity cost including the indirect costs of unnecessary further investigation of healthy individuals following false positive results. This study aimed to evaluate the effectiveness and efficiency of two interventions to modify the test requesting behaviour of general practitioners in Grampian and Moray.

A 2x2 factorial cluster randomised controlled trial design was used. A total of 558 general practitioners (86 practices) were randomised. The two interventions, enhanced educational feedback and test report reminders, were introduced over a one year period. The effect on the requesting behaviour of specific targeted tests was observed across the intervention groups. The overall costs were considered taking into account the costs of delivering the interventions as well as potential savings.

Target test requesting in the control group rose by 5.4% during the intervention period. This compared to falls in test requesting in the feedback group (-2.2%), reminder group (-6.1%) and the group receiving both interventions (-16.8%). Set-up and maintenance costs associated with the interventions compared very favourably with the potential savings that could be made due to reductions in test requesting.

Both test report reminders and enhanced educational feedback are suitable vehicles for the delivery of educational interventions aimed at modifying laboratory test requesting behaviour. Implementation of such into routine laboratory service could lead to substantial benefits for the NHS due to a more rational approach to test use and an overall quality improvement in the diagnostic decision making process.

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Spuriously elevated troponin I results in serum gel tubes

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In a study to investigate the suitability of serum gel tubes for immunoassays two out of 40 normal volunteers (laboratory staff) had high troponin I results. Repeat tests on centrifuged aliquots of the original samples had normal results.

To further investigate this a study was carried out on blood from 28 healthy volunteers.

Blood was collected into paired Starstedt, Becton Dickinson and Greiner gel tubes. An EDTA sample was also taken as a control.

One of each pair of gel tubes was inverted once to mix.

The others were inverted eight times as per IFCC recommendations.

Samples were left for at least 30 minutes and then centrifuged at 1900 g for 10 minutes in a centrifuge set at 20°C. After centrifugation samples were left for a further 30 minutes to allow the gel to cure. All samples were centrifuged within 2 hours.

Troponin I was measured using a Beckman Coulter Access 2. After the assay the remaining serum and EDTA plasma was removed and stored at -20°C in labelled 5 mL polycarbonate tubes.

Two more spuriously elevated results were found in Greiner tubes. Results on centrifuged aliquots of the serum were normal.

We no longer measure troponin I on primary tubes. Secondary aliquots are used which are centrifuged immediately before the assay.