

Posters to be displayed on Wednesday

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A new more effective antiglycolytic agent: a mixture of fluoride oxalate and glyceraldehyde

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Glycolysis is not completely or predictably inhibited post collection by current glucose preservatives, with glucose values falling by as much as 0.5 mmol/L during a 2-4 h period. Immediate centrifugation of all samples is impractical and therefore misdiagnosis of disease can occur, especially if more emphasis is being placed on fasting glucose for the diagnosis of diabetes.

Glycolysis at room temperature was evaluated using glyceraldehyde alone and in conjunction with standard antiglycolytic agents over time. All glucose measurements were performed on an Olympus AU600 analyser using a hexokinase method. The coefficient of variation (CV) of glucose at 3.48 mmol/L was 2.6%. All additives were freeze-dried (to avoid dilutional effects) in amounts such that the desired concentrations would be reached when 1 mL of blood was added. The experiment compared 11 μ mol of sodium fluoride alone, 11 μ mol of glyceraldehyde alone, and the combination of 11 μ mol of sodium fluoride with 11 μ mol glyceraldehyde over 4 time points covering a 24 h period.

Immediately centrifuged samples had glucose concentrations of 6.6 0.1 mmol/L (mean SD). Glucose concentrations were compared using 3 antiglycolytic agents at 0, 3, 8 and 24 h after collection. For sodium fluoride alone, values of 6.57 0.1, 6.27 0.1, 5.93 0.05, 5.97 0.05 mmol/L were obtained for the stated time points. For glyceraldehyde alone the values were 6.77 0.1, 6.33 0.1, 5.27 0.05, 5.3 0.05 mmol/L and for sodium fluoride combined with glyceraldehyde 6.53 0.05, 6.63 0.2, 6.6 0.1, 6.6 0.

Neither sodium fluoride nor glyceraldehyde alone inhibits glycolysis completely. The combination of 11 mmol/L glyceraldehyde, 119 mmol/L sodium fluoride and 21.7 mmol/L potassium oxalate gave the best anti glycolytic results, as glucose concentrations when stored for 24 h were no different from that measured in samples centrifuged immediately. Plasma glucose concentrations obtained from blood collected into tubes containing glyceraldehyde, sodium fluoride and potassium oxalate will more closely reflect those in the patient at venepuncture.

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Prevalence of haemoglobin variants opportunistically identified in the Mid Cheshire population

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The best practice guidelines for the management of diabetes mellitus recommend measurement of haemoglobin A1c (HbA1c) to assess glycaemic control. Measurement of HbA1c is a simple test. However, the presence of a haemoglobin variant in an individual patient may cause problems in the analysis of HbA1c resulting in a falsely low or high value.

The National Institute of Clinical Excellence guidelines suggest maintenance of HbA1c below 6.5% to prevent micro- and macro-vascular complications of diabetes mellitus. Therefore, an accurate analysis of HbA1c is essential.

The Mid Cheshire population is largely white Caucasian. The well-described haemoglobinopathy of the sickle type is rare in this population. However, the uncommon minor variants, which produce minimal effects on a patient's health, may interfere with the final HbA1c result. This is untested in the Mid Cheshire population. The HbA1c by HPLC on the G7 (Tosoh Biosciences) analyser, identifies suspected abnormal haemoglobin bands, which requires confirmation. We wished to confirm that the aberrant bands detected were true haemoglobinopathies.

Methods and Patients: Approval was granted by the local research ethics committee, samples with HbA1c chromatographs showing an abnormal haemoglobin were re-analysed with a specific column (Tosoh Biosciences Specific Thalassaemia) to identify the haemoglobinopathy. All abnormal samples received in a three-month period were analysed using this specific column.

Sixty-two abnormal chromatograms on the HbA1c column were re-assayed and specific abnormal bands identified.

This study suggests that HbA1c on a G7 analyser identifies accurately aberrant haemoglobin bands. Depending on the variant present, a judgement can be made as to the accuracy of the HbA1c result, thus providing a value-added comment to the final report and aiding in the clinical management of a patient with haemoglobinopathy and diabetes.

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Evaluation of a method using dried blood spots as the sample type for HbA1c analysis

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The aim of this study was firstly to determine the stability of dried blood spots when used as the sample type for HbA1c analysis and secondly to establish the accuracy and imprecision of the method.

HbA1c analysis was performed on the Menarini Diagnostics HA 8140 automated haemoglobin analyser, aligned to DCCT/UKPDS values for HbA1c using A.Menarini calibrators.

The blood spot collection paper was obtained from Whatman International Ltd (Catalog number 9535-9362 Grade BFC 1800). 15 µL of whole blood was applied to the paper, the sample was allowed to dry and sent to the laboratory. After storage a 10 mm diameter hole was punched in the paper and the sample was eluted with 1 mL of haemolysant (Menarini Diagnostics product code U8733). The sample's haemoglobin fractions were then analysed using reverse phase ion exchange chromatography on the HA-8140 in pre-haemolysed mode.

Obtained results showed the blood spots were stable for up to 72 hours when stored at room temperature. Excellent correlation ($y=1.01x + 0.06$, $r^2=0.9799$) was obtained between fresh EDTA preserved whole blood results and blood spot results. At a HbA1c level of 5.71% a CV of 1.3% was achieved ($n=20$, $SD=0.074$) and at a HbA1c level of 10.2% a CV of 0.62% was achieved ($n=20$, $SD=0.067$).

In conclusion, this study demonstrated that a method using dried blood spots as sample type can be used for analysis of HbA1c to obtain reliable, accurate and precise HbA1c results.

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Comparison of haemoglobin A1c measurement by the Bio-Rad D-10 analyser with an immunoturbidimetric-colourimetric method on the Beckman LX20

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Haemoglobin A1c (HbA1c) analysis is essential in all hospitals that provide a diabetic service. However, the market is dominated by large through-put analysers, which are not economically viable for smaller laboratories. The choice for smaller laboratories has been limited to devices marketed at the point-of-care community, which have been reported to have poorer precision than many laboratory methods, or the use of

immunoturbidimetric-colourimetric assays that can be used on routine analysers. The new Bio-rad D-10 HbA1c analyser uses the same analytical principle of ion-exchange HPLC as the larger Bio-rad Variant analysers, but is designed to analyse up to 10 samples in a batch, making it ideal for smaller workloads.

We compared our current method on the Beckman LX20 with the D-10. The LX20 method requires an off-board haemolysing step, followed by measurement of total haemoglobin (absorbance at 560 nm), and analysis of the A1c fraction by a turbidimetric immunoinhibition method. The D-10 samples from primary tubes and carries out an onboard dilution before being injected into the analytical flow path; analysis time is 4 minutes. Inter-assay CVs ($n=20$) for the D-10 were 1.66% (5.78%) and 1.42% (10.03%) and for the LX20 were 3.64% (5.68%) and 3.15% (9.46%). However, comparison of data by both linear regression ($n=143$, $r=0.87$, $LX20= 0.95(D10) + 0.76$) and Altman-Bland plot showed significant discrepancies between the 2 methods ($p<0.05$, paired t-test). A positive bias on the LX20 method had also been observed from EQA performance.

The D-10 has documented traceability to the DCCT reference method, and also has the option to use calibrator values traceable to the IFCC reference method. The D-10 also has the advantage of recognising haemoglobin variants, which can cause interference in the immunoturbidimetric assay. The D-10 is a welcome addition to the range of analysers for measurement of HbA1c.

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The introduction of an automated HbA1c method that resulted in improved patient care

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HbA1c is a well established retrospective indicator of glycaemic control in patients with diabetes mellitus. Previously our laboratory used an expensive and time-consuming electrophoresis (Paragon Diatrac) system, more recently we have been using an automated latex agglutination inhibition (Bayer ADVIA 1650) method.

The aim was to compare the two methods and see what benefits, if any, arose from the change in method.

We compared the results of the two analytical methods on peripheral blood obtained from 100 outpatients at the Diabetic clinic at Tygerberg Hospital. We further compared finger prick samples to whole blood EDTA samples on the Bayer ADVIA 1650 on a different group of 38 patients from the same clinic.

We found that the mean HbA1c values obtained (Diatrac 9.3%, ADVIA 10.4%) differ by 1.1% HbA1c. This was reflected in the reference intervals suggested by

the manufactures of each assay (Diatrac 3.8-4.9%, ADVIA 4.5-6.2%). Correlation was 0.82 and the Bland-Altman plot showed a positive bias. The comparison between the finger prick and whole blood samples demonstrated a mean of 10.8% for both sampling techniques, with a correlation of 0.95 and no obvious bias on the Bland-Altman plot.

The overall laboratory time was shortened from a 47 min, batch dependant (minimum of 9 samples) electrophoresis method to a 15 minute, random access, automated assay, improving the turnaround time from a minimum of 285 (median 1464) minutes to minimum of 52 (median 126) minutes, allowing the patient to consult the clinician with a current HbA1c result.

The new method offers many advantages to the diabetic clinic at Tygerberg Hospital, in particular enabling the clinician to attend the patient with a current HbA1c result. The new method provides a suitable HbA1c result, taking into consideration the limitations of the analyte.

16 HbA1c analysis on the HA-8160: nice chromatography, shame about the integration

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In 2003 Menarini won the tender to provide haemoglobin A1c (HbA1c) analysers to the Hammersmith Hospitals Trust (HHT). Five analysers were requested each with an expected workload of 15,000 tests/annum. A phased installation of HA-8160 analysers was completed in December 2003.

One of the attractive features of the HA-8160 is the short analysis time of 2.9 minutes. This is achieved by using reversed phase partition chromatography to elute HbF, labile HbA1c and stable HbA1c, then switching to ion exchange chromatography for the elution of HbA0/A2 and HbS/C.

The chromatogram is also split into two parts corresponding to the different types of chromatography. A different gain allows all the haemoglobins to appear on the same graph. It soon became evident that it is not only the gain that changes after elution of HbA1c. Peak integration shifts to use time windows for HbA0/A2 and HbS/C. In several samples the HbA0 peak clearly comprised two peaks, both were counted as HbA0. On electrophoresis these samples were found to contain haemoglobin variants (usually HbD/E). Inclusion of these variant haemoglobins in the HbA0 peak resulted in falsely low HbA1c results.

As an interim measure we now run one of our five analysers in the β -thalassaemia mode. The enhanced separation allows differentiation of HbA0 from the

variant haemoglobins. In the period from June to December we have performed 22,650 analyses on the HA-8160 and have detected variant haemoglobins (excluding HbS/C) in 53 subjects.

The HHT and Menarini are currently working together to resolve this issue.

17 Routine screening for thyroid dysfunction in Type II diabetic subjects: a seven year follow-up

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Routine annual screening for thyroid dysfunction in Type II diabetic subjects is common clinical practice despite no evidence-based national or international guidelines. With increasing incidence of Type II diabetes, the costs and laboratory workload for thyroid function tests are considerable. The aims were to determine: i) prevalence and annual incidence of thyroid dysfunction, ii) cost effectiveness of routine screening, and iii) if ethnicity is a risk for developing thyroid dysfunction.

978 adults (431 males and 547 females) with Type II diabetes attending hospital diabetic clinic were randomly selected and examined retrospectively for 7 years (1997-2003). Mean age of cohort was 61.9 ± 13.7 years. 385 patients were South Asians, 293 Caucasians, 175 Afro-Caribbeans, 6 Chinese and 119 unspecified. Thyroid antibodies were measured only in subclinical states and if positive, appropriate treatment was provided.

Prevalence of thyroid dysfunction was 19.0%, higher in females (13.6%) than in males (5.4%). Caucasians had highest prevalence (23.9%), Afro-Caribbeans the lowest (8.6%) and the prevalence was 17.4% in South Asians. The most common thyroid disorder was hypothyroidism (10.8%) followed by hyperthyroidism (5.0%), subclinical hypothyroidism (2.5%), and subclinical hyperthyroidism (0.1%). Highest prevalence was in the seventh decade (6.2%).

The average annual incidence (number of new cases excluding subclinical forms) was 1.5% with no excess in any ethnic groups. Although the highest incidence was in the seventh decade (3.3%), as a percentage in individual decades, incidence was maximum in the third decade (17.0%).

We conclude: i) prevalence and incidence of thyroid dysfunction in Type II diabetic subjects is higher than their non-diabetic counterparts, as previously reported, ii) ethnicity does not confer additional risk for thyroid dysfunction, and iii) it would appear reasonable that for maximum yield and cost-effectiveness all new diabetic subjects be screened for thyroid function and antibodies and, targeted if antibody positive.

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How secure is the recorded diagnosis of diabetic ketoacidosis in patients admitted to hospital?

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A benchmark for auditing the quality of diabetic services in the UK is the incidence of diabetic ketoacidosis (DKA) within a hospital's catchment area. Accuracy of these figures is therefore critical. Errors in the recorded diagnosis can occur because of inaccurate diagnosis by medical practitioners or inaccurate interpretation of medical entries by non-medical coders who assign an ICD 10 diagnostic code. The aim of this study is to examine the influence of laboratory testing on the recorded diagnosis of DKA and to establish whether greater availability of plasma ketone measurement might improve the accuracy of the recorded diagnosis.

All episodes coded as DKA between 1998-2003 were identified from our database and a random sample of case-notes were analysed retrospectively by an experienced Specialist Registrar to see whether they fulfilled the diagnostic criteria defined by the American Diabetes Association for DKA. 75/440 (17%) episodes were analysed.

The retrospective analysis revealed that 25(33.3%) episodes were correctly classified and recorded as DKA, 43 (57.3%) had nonketoacidotic hyperglycaemia, 2 (2.7%) had hypoglycaemia, 2 (2.7%) did not have diabetes and 3 (4%) had insufficient data.

Of those that were incorrectly classified as DKA, 17(34%) were incorrectly diagnosed by medical staff and 33 (66%) were miscoded by coders. Of the patients misdiagnosed by medical staff 9 (53%) had ketonuria without acidosis and 13 (76%) were given intravenous insulin on a sliding scale.

This audit suggests that the incidence of DKA was over-estimated in this hospital. The use of capillary blood ketone testing has been shown to be a more specific test for ketoacidosis than urinalysis and may be a useful adjunct to help distinguish it from uncomplicated hyperglycaemia.

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A comparison of cystatin C versus serum creatinine and calculated GFR as markers of renal function in type 1 diabetics

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Detection of early renal dysfunction with appropriate follow-up is an important part of delaying progression to

overt diabetic nephropathy. The aim of this prospective study was to assess the clinical usefulness of cystatin C in comparison with serum creatinine in detecting patients with early renal dysfunction or other diabetic complications.

Thirty-six patients with type 1 diabetes were recruited from the diabetes clinic. Data was collected by questionnaire including number of years since diagnosis, any anti-hypertensive treatment and evidence of nephropathy and/or retinopathy. Serum cystatin C and urinary microalbumin were measured using the BN ProSpec nephelometer (Dade). For comparison serum creatinine was measured and creatinine clearance calculated using the Cockcroft and Gault (C&G) formula. Data was also collected for electrolytes and HbA1c.

Cystatin C correlated significantly with serum creatinine ($y = 0.01x - 0.1268$, $r=0.776$, $p<0.001$) and with calculated GFR ($y = -0.0056x + 1.2564$, $r=0.433$, $p=0.008$). The diagnostic accuracy for detecting patients with significant microalbuminuria (albumin:creatinine ratio >3.0) was assessed by ROC curves. The area under the curve was 0.73 for cystatin C, 0.71 for serum creatinine and 0.65 for estimated GFR respectively.

From this we conclude that cystatin C is a marginally better marker of early renal dysfunction than the traditional serum creatinine but further studies would be required to determine whether it is cost effective. Calculating GFR using the C&G formula did not improve the diagnostic accuracy of serum creatinine in this patient group.

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Investigation of diabetic nephropathy and HLA class II genes: search for a molecular marker

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Diabetic nephropathy affects 30-40% of type I (insulin dependent) diabetic patients. Early therapeutic intervention and the quality of metabolic control is essential for improved survival rates, however genetic factors may also contribute. The HLA-DQB1 gene is involved in the genetic susceptibility to type I diabetes and has been suggested to have a role in the development of diabetic nephropathy. Literature evidence is conflicting. The aim of this study was to determine the association of HLA-DQB1 alleles with diabetic nephropathy, in order to identify a molecular marker.

Type I diabetic subjects ($n=137$) attending the diabetic clinic with IDDM for >10 years had an EDTA blood sample taken for genetic analysis. HbA1c, creatinine and albumin excretion rate (AER $\mu\text{g}/\text{min}$) were measured and duration of diabetes recorded. Diabetic subjects were separated into two groups depending on AER as follows:

Group 1: >30 µg/min, (n=34) and Group 2: patients with AER <20 (n=98), matched for age, disease duration and HbA1c. Data was also compared to normal controls. DNA was purified using a Qiagen DNA mini kit and HLA-DQB1 typed using sequence specific polymerase chain reaction (PCR-SSP).

Frequencies of individual HLA-DQB1 alleles and combinations of alleles (0201, 0301, 0302/5, 0304, 0401/0402, 0501-0504, 0601-0609: 0201/0302/5, 0201/0501-0504, 0201/0601-0609) were compared between groups 1 and 2. In group 1 29% of patients were type 0201/0302/5 and only 3% 0201/0601-0609, however we found no association between HLA-DQB1 alleles and AER (all p values >0.05). There were as expected significant differences between the diabetic group as a whole and the normal controls for HLA-DQB1 allele combinations 0201/0302/5 susceptible (p<0.001) and 0601-0609, protective (p<0.001).

This data further supports the growing evidence that HLA class II genes are unlikely to be of importance in the development of diabetic nephropathy.

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Urinary enzyme measurements as early indicators of renal insult in type 2 diabetes

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The aim of this study was to investigate the association between urinary enzyme activities, namely alanine aminopeptidase (AAP), N-acetyl-β-D-glucosaminidase (NAG) and α glutathione-S-transferase (αGST), and urine microalbumin concentrations in subjects with type 2 diabetes. Thirty-six type 2 diabetic subjects and fifteen age- and sex-matched non-diabetic subjects were recruited. Diabetic subjects, both males and females, were grouped according to their urine protein concentration into those with microalbumin <3 mg/L (group A: 7M/5F), 3-30 mg/L (group B: 5M/7F) and 30-300 mg/L (group C: 6M/6F). Fasting blood and urine samples were taken and analysed for HbA1c, creatinine, AAP, NAG and α GST. HbA1c (%) values [mean(SD)] were 5.2 (0.4), 6.8 (0.7) and 9.1 (1.4) in groups A, B and C respectively and increased with increasing magnitude of microalbuminuria. While serum creatinine concentrations remained within the laboratory reference range (<115 µmol/L), NAG (U/mmol creatinine) activities were significantly elevated in groups A, B and C at 98.7 (8.6), 112.8 (12.9) and 147.4 (16.2) respectively (reference range ≤35 U/mmol creatinine) [group C v groups A and B both p<0.01]. AAP activity (U/mmol creatinine) was elevated in group A [2.6 (0.2)], B [7.6 (0.5)] and C [7.9(0.6)] compared to control group values [2.4(0.2)]

(group C v group A and control group p<0.001). αGST (U/mmol creatinine) activity was also elevated in group A [1.4 (0.1)], B [2.3 (0.4)] and C [2.8(0.5)] compared to control group values [1.1 (0.1)] (group C v group A and control group p<0.001). The results suggest that renal tubular damage in subjects with diabetes may occur prior to glomerular damage. A lysosomal proximal tubular cell enzyme NAG, tubular brush border enzyme AAP and renal proximal tubular cytosolic enzyme; GST measurements may therefore be used to reflect early renal tubular insult in the diabetic state.

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Regulation of pancreatic beta cell AMP-activated protein kinase activity: does metformin inhibit secretion?

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Background and aims: The energy sensing enzyme AMP-activated protein kinase (AMPK) is inhibited by increasing glucose concentrations over the physiological range in malignant beta cell lines (MIN-6). This mechanism is mandatory for glucose-stimulated insulin release. Metformin and leptin have recently been found to activate AMPK in hepatocytes and skeletal muscle. In this study we investigate the effects of metformin and leptin on the regulation of beta cell AMPK with particular focus on insulin secretion in intact rat islets and MIN-6 cells.

Materials and methods: Rat islets were isolated by collagenase digestion and Histopaque gradient centrifugation. The islets were then cultured for one week in RPMI 1640 medium, then 16-20 hours in Dulbecco's MEM containing 3 mM glucose before AMPK activity assays. MIN6 cells (passages 20-30) were maintained in Dulbecco's MEM containing 25 mM glucose, then switched to 3 mM glucose the day before the experiments. AMPK activity was measured against the synthetic peptide SAMS on whole cell extracts or after AMPK immunoprecipitation with anti-AMPK alpha pan antibody using ³¹P

Results: Glucose inhibits total AMPK activity from 100% at 0 mM to 60% at 3 mM and 45% at 17 mM. Metformin increases AMPK activity in a dose responsive manner after an overnight incubation and leptin has no effect on AMPK activity.

Conclusion: Glucose regulates AMPK activity, emphasizing its physiological role in glucose-stimulated insulin secretion. Metformin, a widely used anti-diabetic and insulin sensitising drug, may suppress insulin secretion by activating AMPK.

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Maternal plasma leptin levels and their relationship to insulin and glucose in gestational diabetes mellitus and gestational impaired glucose tolerance

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The aim of the study was to investigate the changes in plasma leptin levels and their relationship to insulin and glucose in pregnant women with gestational diabetes mellitus (GDM) and gestational impaired glucose tolerance (GIGT).

A 75 g oral glucose tolerance test was performed in 50 pregnant women at 24-28 weeks of gestation. 25 of which were women with gestational diabetes and 25 normal pregnant women. In each pregnant woman, fasting and 2 h plasma leptin, insulin and glucose levels were measured. Enzyme-linked immunoassay was used to measure the leptin levels.

The fasting and 2 h plasma leptin levels in pregnant women with GDM and GIGT were significantly higher than normal pregnant women by 85% and 77%, respectively. The levels of fasting serum insulin and plasma glucose levels were also significantly higher in pregnant women with GDM and GIGT by 57% and 36%, respectively, as compared with normal pregnant women. Similarly, the 2 h plasma insulin and glucose obtained after oral administration of 75g glucose were significantly higher in pregnant women with GDM and GIGT by 105% and 71%, respectively, as compared with normal pregnant women. A positive correlation was found between maternal plasma leptin levels and fasting plasma insulin levels in pregnant women with GDM and GIGT. A positive correlation was also found between maternal leptin concentrations and fasting plasma glucose levels but not with the 2 h serum glucose obtained after OGTT in women with GDM and GIGT.

Leptin levels are elevated in pregnant women with GDM and GIGT compared with healthy pregnant women, a positive and significant correlation was found between the maternal leptin levels and fasting insulin levels, as well as with fasting plasma glucose levels in women with GDM and GIGT.

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Xenotransplantation as a potential strategy to reverse diabetes

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The aim of the study was to assess the acceptance of xenotransplantation as a potential treatment strategy to reverse diabetes. The number of people with type II

diabetes (diabetes) around the world is increasing. In England alone, there are more than one million people diagnosed with diabetes, with an estimated one million undiagnosed, and the number continues to rise.

The lack of tissue/organs for transplantation is a global problem. However, other potential solutions do exist such as xenotransplantation, the manipulation of human embryonic stem cells and the creation of artificial organs.

The bioethics of xenotransplantation has not been widely discussed in the scientific realm. The major ethical concern is that a treatment at an individual level may have dire consequences at a societal level, such as those arising from the transmission of cross-species pathogens. It is essential that the benefits to society outweigh the risks.

Ethical discussion about xenotransplantation must include justice, respect for autonomy, non-maleficence and beneficence. There is no previously reported data on the acceptance of xenotransplantation versus allotransplantation amongst diabetic patients. Approval from Local Research Ethics Committees was obtained to distribute questionnaires in Australia and England.

Information was gathered from respondents (patients with diabetes mellitus) who attended clinics in Australia and England. Data from the questionnaires (196 Australia and 124 England) was analysed using Excel.

Results: 77% (95) respondents from England would accept human tissue compared to 86% (168) from Australia. 62% (77) would accept pig tissues in England compared to 70% (138) for Australia. Non-human primate tissue would be accepted by 60% (74) in England, compared to 68% (133) in Australia.

The majority of respondents would accept xenotransplanted tissue to reverse their diabetes. Further questionnaires have been distributed in England. Additional work is required to confirm these findings.

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The roles of vitamin A, E and β -carotene as anti-oxidants in diabetes mellitus

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The diabetic patient has an increased susceptibility to the risk of oxidative damage by free radicals. Protection against such damage can be offered by radical-scavenging antioxidants. Such molecules include the lipid soluble Vitamins A, E and β -carotene, which readily scavenge free-radicals thereby protecting important biological moieties from oxidative damage. The study investigated the serum concentrations of Vitamins A, E and β -carotene in 95 diabetic and 30 control subjects, employing reverse-phase high performance liquid

chromatography. The effect of smoking on the vitamin levels, in both the control and diabetic cohorts and the effects of glycaemic control in the diabetic group were also investigated.

Pearson correlation analysis showed that Vitamins A and E had a direct relationship ($n=30$, $r=0.528$, $p=0.03$ in non-diabetics (control); $n=95$, $r=0.539$, $p\leq 0.001$ in diabetics). β -carotene levels did not show any relationship with Vitamins A or E concentrations. No significant difference was observed for [Vitamins E] and [A] in the diabetic and control groups and sub-groups investigated (Mann-Whitney test). A significant difference ($p=0.05$) was observed for [β -carotene] between diabetic smokers ($n=17$, mean= 75.71 $\mu\text{mol/L}$) and diabetic non-smokers ($n=78$, mean= 130.64 $\mu\text{mol/L}$). There was no significant difference ($p=0.207$) in [β -carotene] between poorly (HbA1c $>7.5\%$, $n=60$, mean=106.3 $\mu\text{mol/L}$) and well (HbA1c $\leq 7.5\%$, $n=35$, 145.6 $\mu\text{mol/L}$) controlled diabetics. To ascertain whether smoking alone could be a factor in reduced [β -carotene], statistical comparison (Mann-Whitney test) between non-diabetic smokers ($n=7$, mean=174.6 $\mu\text{mol/L}$) and non-smokers ($n=22$, mean=143.6 $\mu\text{mol/L}$) showed no statistically significant difference ($p=0.925$). However, the number recruited for the non-diabetic non-smokers were low. These results suggest that glycaemic control alone has no significant effect on [β -carotene], [Vitamin E] or [A] in diabetic patients. However, smoking does appear to cause a significant reduction in [β -carotene] diabetic patients. This may be as a result of the additive effects of oxidative stress associated with both diabetes and smoking.

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Biochemical markers of oxidative stress in type 2 diabetes

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Free radical-mediated oxidative stress has been implicated in the pathogenesis of diabetes and its complications. Oxidative stress has been defined as a disturbance in the balance between the production of free radicals and antioxidant defenses.

The aim of the study was to compare the lipid peroxidation marker urinary thiobarbituric acid reactive substances (TBARS) and antioxidants including plasma α -tocopherol (vitamin E), both plasma (P-GHS-Px) and erythrocyte glutathione peroxidase (E-GHS-Px) and plasma selenium levels in type 2 diabetic subjects ($n=30$) and non-diabetic age and gender-matched control subjects ($n=40$). The duration of diabetes was from 4-6 years. Both males and female diabetic subjects attending

the diabetic clinic were randomly selected and a fasting blood and MSSU samples obtained from both diabetic and non-diabetic subjects. HbA1c, urine TBARS, P-GHS-Px, E-GHS-Px and plasma selenium concentrations were measured.

HbA1c (%) levels were elevated in all diabetic subjects [12.3 (1.1)] [mean (SD)]. Elevated urinary TBARS ($\mu\text{mol/mmol creatinine}$) level was noted in diabetic subjects [2.8(0.3)] compared to control subjects [1.1(0.5)] ($p<0.001$). A significant correlation was noted between HbA1c level and TBARS ($r=0.894$; $p<0.01$). Plasma vitamin E levels ($\mu\text{mol/L}$) were significantly decreased in the diabetic group [18.9 (2.1)] compared to control group values ($p<0.001$). P-GHS-Px (U/L), E-GHS-Px (U/g Hb) activities and plasma selenium levels (mol/L) were also decreased at 4800 (43), 51.3 (4.9) and, 1.6 (0.1) respectively (all $p<0.01$). Non-diabetic control group values remained within the laboratory reference range for all antioxidant parameters measured.

These observations further support the suggestion that chronic hyperglycaemia can influence the generation of free radicals that may ultimately lead to increased lipid peroxidation and depletion of antioxidants and thereby enhanced oxidative stress in subjects with type 2 diabetes mellitus.

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Inflammatory markers in type 2 diabetes

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Type 2 diabetes (T2D) is associated with a significant increase of cardiovascular mortality and accelerated atherosclerosis. Recent evidence has linked acute phase reactants to the increased risk of micro- and macrovascular complications in diabetes. We therefore performed a cross-sectional study of patients with T2D measuring a profile of inflammatory markers and relating them to the presence of cardiovascular risk factors.

CRP, TNF-alpha, interleukin-6 (IL-6), interleukin-8 (IL-8) beside a full lipid profile and HbA1c were measured in serum of 53 patients with T2D (age range: 45-79) and 29 controls (age-range: 20-52). Anthropometric parameters (weight, height, waist-circumference, blood pressure) were taken and presence or absence of hypertension and coronary heart disease documented.

CRP and IL-6 were significantly higher in the T2D group even after allowing for age, BMI and waist circumference (mean CRP 3.16 mg/L vs 1.8 mg/L; mean IL-6 3.99 pg/ml vs. 1.14 pg/ml, $p<0.05$). CRP, IL-6 and TNF-alpha in the T2D group were all positively correlated with serum cholesterol and cholesterol/HDL ratio respectively ($r=0.33$, $r=0.51$, $r=0.31$, $p<0.05$), while only CRP was positively correlated with glycaemic

control ($r=0.29$, $p<0.05$). Presence or absence of hypertension and coronary heart disease did not influence the level of cytokines or CRP. However, when categorized for the presence or absence of the metabolic syndrome, CRP and IL-6 were significantly higher in subjects with the metabolic syndrome.

In conclusion, we have demonstrated increased levels of CRP and IL-6 in T2D, but in contrast to other studies TNF-alpha and IL-8 were not significantly raised. Inflammatory markers were positively associated with an adverse lipid profile and subjects with the metabolic syndrome expressed the highest levels of CRP and IL-6.

28 Homocysteine and sensitive-CRP are associated with microalbuminuria in patients with type 2 diabetes

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Homocysteine and sCRP are established risk factors for cardiovascular disease (CVD) and are elevated in the presence of renal impairment. The effect of microalbuminuria on homocysteine and sensitive-CRP levels in patients with diabetes has not been fully characterised. Microalbuminuria in type 2 diabetes is known to be associated with increased CVD risk. The combination of renal impairment and hyperhomocysteinaemia may confer additional risk, especially in patients with type 2 diabetes. This study examines the association of these factors with microalbuminuria and investigates their use in the clinical environment. Samples were collected from 110 patients with type 2 diabetes for analysis of homocysteine and sCRP as part of their annual review at the diabetes clinic. CRP levels below 5 mg were measured using a high-sensitivity CRP assay. The presence of microalbuminuria was assessed on early morning urine samples. Patients with significant renal impairment were excluded from the study. Patients with microalbuminuria had a significantly raised homocysteine level compared to those without (17.2 ± 6.9 mmol/L, 11.7 ± 6.3 ; mean \pm SD; $p<0.01$). sCRP was also higher in patients with microalbuminuria than those with normal renal function (3.56 ± 0.78 mg/L, 1.87 ± 0.98 , $p<0.05$). Microalbuminuria is associated with increased homocysteine and sCRP levels. This may, in part, explain the increased CVD risk of these patients. We recommend the measurement and treatment of elevated homocysteine levels in type 2 diabetic patients with microalbuminuria.

29 Homocysteine and other markers of cardiovascular disease in a population with type 2 diabetes

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Patients with type 2 diabetes have a greatly increased cardiovascular disease (CVD) risk. This is not wholly accounted for by conventional CVD risk markers. Other novel risk factors include, homocysteine, apoA1, apoB and sensitive-CRP. The role of these analytes in patients with diabetes has not been fully characterised. This study examines the association of such factors with CVD and investigates their potential for inclusion in risk profile calculations. Samples were collected from 110 patients with type 2 diabetes for analysis of homocysteine, apoA1, apoB and sCRP as part of their annual review. CRP levels below 5 mg were measured using a high-sensitivity CRP assay. CVD risk assessment was performed using the PROCAM model, which generates a percentage risk of a cardiovascular event within the next 10 years. In general, this population had a high level of homocysteine (14.3 ± 6.6 mmol/L; mean \pm SD) which was significantly higher in males (15.9 ± 7.2) compared to females (12.7 ± 5.6 , $p=0.01$). Homocysteine was positively correlated with the percentage risk estimates from the PROCAM model ($11.9\pm 9.0\%$, $p<0.01$). ApoB (68.2 ± 25.7 mg/dL), the apoB:A1 ratio (0.60 ± 0.22) and sCRP (2.21 ± 0.98 mg/L) were also positively correlated with CVD risk ($p<0.05$). Elevated homocysteine, apoB and sCRP levels are associated with increased CVD risk. We suggest that the inclusion of these analytes in the risk model for patients with type 2 diabetes would improve its predictive accuracy.

30 Prevalence of diabetes mellitus in Northern Iraq: comparison of 1997 American Diabetes Association classification with 1985 World Health Organization classification

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Objective: To estimate the overall prevalence of diabetes mellitus (DM) and impaired glucose tolerance (IGT) in Mosul city in Northern Iraq.

Methods: The survey involved screening for DM among a population of 1015 subjects aged ≥ 25 years from different urban and rural areas in Mosul city in Northern Iraq, during the period from 1st August 2000 to 31st January 2001. Fasting plasma glucose (FPG) and 2 hours plasma glucose (2hPG) was measured. The diagnosis of DM was achieved based on both the new 1997

American Diabetes Association (ADA) criteria and the old 1980-1985 World Health Organization (WHO) criteria. The subjects were classified into 5 groups. Group 1: subjects with FPG <6.1 mmol/L (n=883). Group 2: subjects with impaired FPG 6.1-6.9 mmol/L (n=29). Group 3: new diabetics diagnosed solely by new 1997 AD criteria with FPG 7.0-7.7 mmol/L (n=20). Group 4: new diabetics diagnosed according to old WHO criteria with FPG \geq 7.8 mmol/L (n=23). Group 5: known diabetics (n=60). Subjects in groups 2 and 3 underwent a standard 75 gm oral glucose tolerance test (OGTT) as recommended by the WHO. Reclassification of subjects into 3 groups according to FPG and/or 2hPG was done. Group A (non-diabetics): subjects with FPG <6.1 mmol/L and/or 2hPG <7.8 mmol/L (n=910). Group B (diabetics): subjects with FPG \geq 7.8 mmol/L and/or 2hPG \geq 11.1 mmol/L (n=92) including 60 known diabetics in group 5 and 23 new diabetics in group 4 in addition to 2 subjects in group 2 and 7 subjects in group 3. Group C (IGT): subjects with 2hPG between 7.8-11.1 mmol/L (n=13).

The overall prevalence of DM using WHO criteria was 9.1%, however, it rose to 10.35% when the ADA criteria was used. The overall prevalence rate of IGT was 1.28% when the WHO criteria were used and the prevalence of impaired fasting glucose was 2.86% when the ADA criteria were used. To show the effect of age on the prevalence of DM, the subjects were divided into those aged: 24-34, 35-44, 45-54, 55-64 and \geq 65 years of age. The prevalence of DM increased with increasing age. Using the WHO criteria, the prevalence rates of DM in the five groups were 0.31%, 4.33%, 17.33%, 18.67% and 16.85% respectively. When the new ADA criteria were used, the prevalence rates of DM were 0.31%, 5.19%, 18.81%, 21.69% and 20.22% respectively. There was also a significant difference in the prevalence rate of DM in subjects aged <35 years and subjects aged \geq 35 years ($z=18.57$, $p<0.0001$). The prevalence rates of DM and IGT rose from 0.31% and 0.61%, in those <35 years to 13.23% and 1.60% respectively for those aged \geq 35 years old (when WHO criteria was used) and from 0.31% and 0.61% for DM and IGT respectively to 15.12% and 3.49% (when the new ADA criteria were used).

Conclusion: Diabetes mellitus is common in the Iraqi population, and its prevalence rate increases with age.

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Performance indicators and validity of serum fructosamine assay as a diagnostic test in a screening programme

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Objectives: To evaluate the performance indicators and validity of fructosamine assay as a diagnostic tool in

screening for diabetes mellitus (DM).

Methods: Fasting plasma glucose (FPG) and serum fructosamine (FA) were compared in 1015 subjects aged \geq 25 years from different urban and rural areas in Mosul city in Northern Iraq. The subjects were classified into 5 groups. Group 1: subjects with FPG <6.1 mmol/L (n=883). Group 2: subjects with impaired FPG 6.1-6.9 mmol/L (n=29). Group 3: new diabetics diagnosed solely by new 1997 American Diabetes Association (ADA) criteria with FPG 7.0-7.7 mmol/L (n=20). Group 4: new diabetics diagnosed according to old 1980-1985 World Health Organization (WHO) criteria with FPG \geq 7.8 mmol/L (n=23). Group 5: Known diabetics (n=60). Subjects in groups 2 and 3 underwent a standard 75 gm oral glucose tolerance test (OGTT) as recommended by the WHO. Reclassification of subjects into 3 groups according to FPG and/or 2hPG was done for all subjects. Group A (non-diabetics): subjects with FPG <6.1 mmol/L and/or 2hPG <7.8 mmol/L (n=910). Group B (diabetics): subjects with FPG \geq 7.8 mmol/L and/or 2hPG \geq 11.1 mmol/L (n=92) including 60 known diabetics in group 5 and 23 new diabetics in group 4 in addition to 2 subjects in group 2 and 7 subjects in group 4. Group C (impaired glucose tolerance [IGT]): subjects with 2hPG between 7.8-11.1 mmol/L (n=13).

Results: All subjects had their serum FA measured and the Receiver Operator Characteristic (ROC) curve was constructed on the data to determine the trade-off between sensitivity and specificity. This construction decided that serum FA value of 2.65 mmol/L would be the cut-off point or the positivity criterion in the calculation of the validity parameters of FA test. Of 910 non-diabetics, 886 subjects had measured FA values within the 95th percentile, while 24 had FA higher than the cut-off point. Consequently, FA in non-diabetics yielded 886 true negatives and 24 false positives. Accordingly, the sensitivity, specificity, positive predictive value, negative predictive value, accuracy rate, positive likelihood ratio and negative likelihood ratio were 67.3%, 97.3%, 72.3%, 96.7%, 94.6%, 26 and 2.99 respectively. A highly significant correlation was observed between FPG and measured FA in non-diabetics ($r=0.85$, $p<0.0001$) and diabetics ($r=0.92$, $p<0.0001$). No significant correlation with observed between serum FA and albumin in non-diabetics ($r=0.14$, $p>0.05$) and diabetics ($r=0.08$, $p>0.05$).

Conclusion: Fructosamine shows a moderate sensitivity with a high specificity as a diagnostic test for diabetes mellitus. The considerable overlap between diabetics and non-diabetics limit its usefulness. It is recommended that fructosamine test is not a suitable screening text for the disease. Measurement of plasma glucose (fasting or post-OGTT) remains the corner stone as a diagnostic test.

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Audit of the diagnosis of diabetes in inpatients

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Plasma glucose is routinely requested in many patients admitted to hospital. Previous studies in the UK and USA have shown that most results were not acted upon or were overlooked. New diagnostic criteria for the diagnosis of diabetes mellitus (DM) were implemented in 2000. The cut off points for diagnosis are: fasting plasma glucose (FPG) ≥ 7.0 mmol/L or random plasma glucose (RPG) ≥ 11.1 mmol/L. If RPG is between 5.5 and 11.0 mmol/L, repeat after fasting is recommended. The aim of this audit was to assess whether plasma glucose results were acted upon and, whether the new criteria were being followed up by physicians.

One hundred inpatients, in whom plasma glucose was measured, were randomly selected from the laboratory computer. After excluding known diabetics and those who died while in hospital, 86 patients were included in the audit.

Of the 86 patients, 7 patients had plasma glucose values diagnostic of DM (all 7 had RPG ≥ 11.1 mmol/L) and 13 patients had plasma glucose levels of ≤ 5.5 mmol/L. Of the remaining 66 patients, who had RPG between 5.5 and 11.0 mmol/L, only 8 patients were followed up by having plasma glucose repeated after fasting. Of the 7 patients who had values diagnostic of DM, only 2 (29%) were diagnosed as having DM.

Our audit shows that plasma glucose results on inpatients are not acted upon and that the new criteria for diagnosis of DM are not being followed. The majority of patients (71%) with hyperglycaemia, diagnostic of DM, were discharged without documentation. Of those who should have been followed up with a fasting glucose, only 12% were followed up. Printing the diagnostic cut off points for diagnosis of DM by laboratories, when reporting plasma glucose, may help to improve the early diagnosis and treatment of diabetes mellitus in inpatients.

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Impact of changing the glucose load on the diagnosis of gestational diabetes mellitus

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We undertake universal screening for gestational diabetes mellitus (GDM). All women undergo a Lucozade challenge at 28 weeks and an OGTT if the 1-hr plasma glucose (1hr PG) is above 7.8 mmol/L. We diagnose GDM

if the OGTT 2hr PG is above 8.9 mmol/L with or without a fasting plasma glucose (FPG) above 5.3 mmol/L. In 2002 we realised that the glucose load we were using contained 75 g of dextrose monohydrate instead of 82.5 g (equivalent to a 75 g glucose load). We audited the impact of correcting this error on the diagnosis of GDM.

In the 12-month period prior to the change a total of 1024 OGTTs were undertaken in our Maternity Hospital. The number of OGTTs with abnormal results was 92/101 based on FPG/2hr PG criteria respectively. In the 12-month period following the change 1228 OGTTs were undertaken and 119/173 had abnormal results. The proportion of abnormal FPG results did not differ pre versus post change (9.0 vs 9.7% $p=0.57$). Abnormal 2hr PG results increased significantly from 9.9 to 14.1% ($p=0.0011$). We understand from the supplier that many Trusts use the incorrect amount of dextrose monohydrate for OGTTs. Our data indicate that this leads to many missed diagnoses of GDM.

The total number of deliveries undertaken during the 2 time periods was 4539 and 4821 giving an overall prevalence of GDM in our population of 163/4539 (3.6%) pre-change and 246/4821 (5.1%) post-change. This represents a 44% increase in workload for our diabetes-in-pregnancy team.

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Oral glucose tolerance testing in the community

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The OGTT remains the definitive test for diabetes mellitus. In order to deal with an increasing number of requests the decision was taken to introduce the OGTT into the community. This was facilitated through the outpatient department of a community hospital in Liverpool, Clinical Chemistry providing the analytical and interpretive service.

An audit showed that the method of performing the OGTTs did not always follow WHO guidelines. The community hospital was suitably advised and the impact on diagnostic rates was subsequently determined with a repeat audit. A comparison was made with the OGTTs performed in the Programmed Investigation Unit (PIU) of the RLUH.

The pre-change audit involved 134 patients within the community and 261 within the PIU. There was no significant difference between the diagnostic classification rates based on fasting ($p=0.52$) or 2-hour glucose concentration ($p=0.12$) of the two groups. There was, however, a significant difference ($p=0.01$) in the numbers of normal patients in the two groups showing a 2-hour concentration significantly less ($p<0.001$) than the

fasting concentration (31% of community patients, 20% of PIU patients). The latter group of patients generates a number of queries from GPs.

The post-change audit involved 105 patients within the community and 176 within the PIU. Again, there was no significant difference between the diagnostic classification rates based on fasting ($p=0.38$) or 2-hour glucose concentration ($p=0.21$) of the two groups. Following the procedural changes however, there was now no significant difference ($p=0.54$) between the numbers of normal patients in the two groups showing a 2-hour concentration significantly less ($p<0.001$) than the fasting concentration (25% of community patients, 22% of PIU patients).

This audit has demonstrated that community-based OGTTs are a viable option but continuing education and training is required for this to be successful.

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The utility of HbA1c to predict an abnormal oral glucose tolerance test

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Oral glucose tolerance test (oGTT) is a standardised test recommended by the World Health Organisation (WHO) for the laboratory diagnosis of diabetes mellitus (DM). Failure to follow the correct protocol may lead to misclassification or misdiagnosis. oGTT is inconvenient to patients, requiring preparation (fasting) and attendance at hospital outpatients or GP surgeries. A random blood sample for haemoglobin A1c (HbA1c) is a simple procedure, with little inconvenience to patients. HbA1c provides a weighted moving average of the last three months exposure to glucose. We investigated the utility of HbA1c to predict the outcome of an oGTT.

We describe a retrospective analysis of oGTT and HbA1c tests performed in our laboratory. 1322 patients attended the laboratory in 2002 for oGTT of which 328 patients also had HbA1c. The blood sample for HbA1c was collected within 2 months of the oGTT. Data was analysed using the Statview (Oxford) Statistical Software.

Using the American Diabetes Association and the revised World Health Organisation criteria, 28 patients showed a non-diabetic (ND) response, 35 impaired fasting glycaemia (IFG), 98 impaired glucose tolerance (IGT) and 167 showed a diabetic (DM) response to a glucose challenge. There was significant correlation between HbA1c between ND/DM ($p\leq 0.001$) and ND/IGTT ($p\leq 0.001$) and ND/IFG ($p=0.001$). At a cut-off point of HbA1c = 6.0%, we have 96% specificity and 49% sensitivity to detect fasting hyperglycaemia. Of note, 20 (4%) patients missed at HbA1c = 6.0%, all had mildly

impaired fasting glycaemia.

We conclude that HbA1c without oGTT could be used to diagnose patients with suspected diabetes. Patient and staff inconvenience can be minimised by use of HbA1c. However, these preliminary results need validation by further large scale studies.

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An audit of the use of OGGT by General Practitioners

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WHO, IFD and ADA guidelines have defined diabetes mellitus on the basis of fasting plasma glucose (FPG) and oral glucose tolerance tests (GTT). This study has audited the use of FPG and GTT by General Practitioners in the York area against their guidelines over a three years period (2001-2003).

All test results of FPG and GTT that have been requested from general practice were collected using the LIMS. In addition, previous glucose measurements on 190 of this group of patients were retrieved from the LIMS. GTT to diagnose gestational diabetes mellitus cases were excluded.

GTT data on 1000 patients were collected. GTT workload increased by 100% from 2001 to 2002 and 2003. FPG was diagnostic in 67.4% and progressing to a 2-hour glucose tolerance test was unnecessary. Of 190 patients, 27.5% had no previous glucose measurements, and 13.5% had previous plasma glucose results, which were either normal or clearly diabetic and were not in compliance with WHO or IFD guidelines.

Our audit of the use of GTT by primary care has shown that this investigation is inappropriately requested in 41% cases due to lack of previous glucose measurement or clearly normal or abnormal baseline glucose measurement

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A case of paediatric post-operative low calcium and alkaline phosphatase activity

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SA, a 4 month old child with Down's syndrome, was admitted to hospital for a congenital atrioventricular septal defect repair. Pre-operative serum urea was 4.8 mmol/L, creatinine 62 $\mu\text{mol/L}$. On day 1, post-surgery his serum adjusted calcium was 2.44 mmol/L and alkaline phosphatase (ALP) 193 IU/L [reference range 100-300 in adults]. He was started on total parenteral nutrition (TPN). On day 4 urea was 9.3 mmol/L, creatinine 204 $\mu\text{mol/L}$. From day 5 onwards he developed

diarrhoea, pyrexia, bilateral pleural fluid effusion and renal failure requiring dialysis.

By day 18 his adjusted calcium had fallen to 1.77 mmol/L, ALP 44 IU/L. The day 17 serum magnesium was 0.82 mmol/L, falling to 0.66 mmol/L on day 20. Despite calcium and magnesium supplementation, day 22 adjusted calcium and ALP rose to only 2.02 mmol/L and 74 IU/L respectively. Further investigation showed serum PTH 150 ng/L [11-55 in normocalcaemic patients], 25-OH vitamin D 12.1 µg/L [10-45], 1,25 di-OH vitamin D 126 pmol/L [20-120] and urine calcium/creatinine ratio 0.131 mmol/mmol. At the time SA was undergoing daily removal of 25 to 50 mL of pleural fluid by aspiration, (equivalent to approximately 6 to 12% of circulating blood volume). The calcium concentration of this was 1.14 mmol/L.

Day 18 serum zinc was 6.2 µmol/L [12.6-22.0]. For the next 2 weeks the TPN zinc and calcium content was increased and magnesium supplementation was continued. Day 30 serum results were: zinc 10.2 µmol/L, magnesium 0.85 mmol/L, adjusted calcium 2.27 mmol/L, ALP 242 IU/L.

Although it is well recognized that cardiac surgery, dialysis and hypomagnesaemia can cause a decrease in serum calcium and ALP we believe, in this multifactorial case, that pleural fluid loss of calcium was a contributory factor to the hypocalcaemia and that the persistent low ALP was probably exacerbated by zinc deficiency.

38 Iatrogenic copper deficiency following information and drugs obtained over the Internet

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We report the case of a 56-year-old lady with a seven year history of metastatic cancer who presented with severe copper deficiency following treatment with the copper-chelating agent, tetrathiomolybdate. This compound was used with the aim of inhibiting tumour angiogenesis. It was obtained on prescription from the USA via the internet. The goal of this treatment was to deplete copper so that caeruloplasmin levels would fall to 20% of baseline levels. Serum copper concentration was assessed by flame atomic absorption on a Unicam SP9 (Philips Pye). caeruloplasmin was assessed by rate nephelometry using a Beckman Coulter Image (Beckman Coulter) The patient exhibited severe neutropaenia as her serum copper concentration fell from 19.8 µmol/L to 3.3 µmol/L and her Caeruloplasmin concentration from 35 mg/dL to 4 mg/dL. This is the first reported case of iatrogenic copper deficiency due to the ingestion of

tetrathiomolybdate. This case raises issues of concern about the evidence base for this line of treatment and medical advice over the internet.

39 An unusual case of poisoning

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A 56 year-old lady presented in A&E as ?overdose at 15.30 h on 19/02/03; she had taken three overdoses in the last 3 weeks and had a long history of psychiatric problems. She denied taking any drugs other than her husband's Rabeprazole, but later refused to answer any questions. On admission her GCS was 13/15 but fell to 8/15 and then 3/15 by 18.00h. At that time she had a metabolic acidosis (pH=7.24) with Na=151, Cl=115 mmol/L.

A&E staff believed she had also taken valproate; the acidosis and her overall condition worsened, and was transferred to ITU. During the next 20 hours pH fell to 6.76, sodium rose to 188 mmol/L, calcium fell from 2.32 to 1.52 mmol/L and creatinine rose from 82 to 261 µmol/L. The osmolar gap was found to be 135 and an overdose of ethylene glycol was considered.

The lethal dose of ethylene glycol is about 100 ml for an adult. It is metabolised to glyoxylic and oxalic acids, which cause cardiopulmonary and renal damage, and high glycolate levels contribute to the osmolar gap. The half-life of ethylene glycol is 3 hours and treatment must be started as soon as possible after an overdose. In this case the diagnosis was made too late to save the patient.

At post-mortem calcium oxalate crystals were visible in the brain and the kidney. Ethylene glycol levels in the admission blood sample were found to be 6.7 mmol/L (toxic level >0.5 mmol/L). The patient's husband later found anti-freeze to be missing from the garage.

Although a rare cause of poisoning, ethylene glycol overdose requires prompt treatment. Osmolality is a useful investigation in investigating possible poisoning by an unknown compound and if measured on admission could have led to prompt treatment of this patient.

40 Whose TPMT activity is it anyway?

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Defining patients who have deficient thiopurine S-methyltransferase (TPMT) activity and so at risk of severe complications and even death if treated with standard doses of thiopurines, is increasingly being used in clinical practice.

Patient MW was commenced on azathioprine to treat limbic encephalitis. She was initially started on a dose of

25 mg/day, this was then increased to 125 mg/day. Two months after azathioprine was started MW developed neutropenic sepsis and staphylococcal skin impetigo. She had become pancytopenic and a bone marrow examination showed severe aplastic anaemia. Azathioprine was stopped after nine weeks treatment. One week later her red blood cell (RBC) TPMT activity was measured and found to be low at 16 nmol 6-MTG/g Hb/hour (normal reference interval 24 to 55 nmol 6-MTG/g Hb/hour).

MW made a good recovery and her blood counts improved. At review eight weeks later her TPMT activity was measured again and found to be non-detectable, ≤ 2 nmol 6-MTG/g Hb/hour. Her TPMT activity status was confirmed using a repeat sample, and by TPMT genotyping. MW was found to be homozygous for TPMT*3A, the most common mutation leading to TPMT deficiency in Caucasians, demonstrating a clear genotype to phenotype correlation.

Due to the discrepancy between the initial TPMT activity result obtained and subsequent results, the clinical history for MW was revisited.

MW had received a blood transfusion of 1 unit blood and 2 units of platelets 6 days before the first TPMT measurement. As the TPMT activity of an individual is assessed using RBC, patients who have received a recent blood transfusion can give misleading results. This is the likely reason the first sample having a measurable TPMT activity, then undetectable activities on repeat analysis.

This case underlines the importance of interpreting TPMT activity status cautiously in patients who have received a recent blood transfusion.

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Transient thyrotoxicosis post parathyroidectomy

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A 70 year old woman was diagnosed with mild primary hyperparathyroidism and underwent surgery for a total parathyroidectomy. At operation she was noted to have a previously undiagnosed thyroid nodule and this was also excised. On day 1 post-operatively routine biochemical investigations were requested including thyroid function tests and these were markedly abnormal. She had been biochemically euthyroid at pre-admission clinic two weeks previously, with no signs or symptoms of thyrotoxicosis. On day one post-operatively the FT4 rose markedly and the TSH fell. By day four the patient was biochemically hyperthyroid TSH ≤ 0.01 mU/L (0.4-4.0); FT4 >77 pmol/L (10-25) although no symptoms attributable to hyperthyroidism were documented in the notes. These abnormalities gradually resolved over the following months although a re-bound rise in TSH was noted 4 weeks post-operatively. FT3 was later measured

on the post-operative samples and found to be high peaking on the first post-operative day at 31.8 pmol/L (4.0-7.8) and returning to normal by day 10.

The operative notes stated that neck exploration was extensive and prolonged. Manual manipulation of the thyroid gland appears to be the only explanation for this patients transient thyrotoxicosis.

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An unusual case of pituitary macroadenoma

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Pituitary tumours secreting prolactin are the most common intracranial tumours. Macroadenoma secreting other pituitary hormones are rare. We present a case of a 44 year old woman with a rare pituitary tumour secreting follicle stimulating hormone (FSH). The patient presented to the ophthalmologist with visual loss. She had regular periods, had no galactorrhoea and no nocturia. On examination she had thin skin, plethoric facies, a BMI of 39 and mild proximal muscle myopathy. Initial investigations showed a raised prolactin (1592 mu/L) FSH of 71.9 U/L, LH of 1.5 U/L and oestradiol of 905 pmol/L. A screening test for heterophilic antibody for FSH, LH and prolactin were negative. The prolactin level after PEG precipitation was 840 mu/L confirming interference from macroprolactin and hence analytical interference in the assays were excluded. Serum growth hormone, cortisol, free thyroxine and TSH were all within normal limits. These results are biochemically consistent with tumour secreting FSH. A CT scan of the head showed a pituitary macroadenoma. Visual field examination showed left side extreme heminopia. Transphenoidal hypophysectomy was performed and post-operatively FSH and prolactin level fell. Gonadotrophinomas are very rare tumours and are more common in middle aged men. The most usual presenting symptom is visual disturbance. Gonadotrophinomas predominately secrete FSH often accompanied by the excessive production of alpha-subunits. Hypopituitarism can occur as a result of compression of secretory cells by the tumour bulk. Raised prolactin, as seen in this patient, is probably due to stalk compression interfering in dopamine secretion

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An LH-secreting adenoma?

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A 33 year old female was referred to the Gynaecology Department complaining of secondary infertility. She has a daughter born by lower segment caesarian section in 2001 and had been trying to conceive for over a year.

Her BMI was 18.4 kg/m², with no excessive exercise. She has the same partner who is well and does not smoke. Semen analysis was essentially normal.

She has a regular cycle of 28 days. Abdominal, vaginal and uterine examination were normal as were a diagnostic laparoscopy, hysteroscopy and dye studies.

The patient had a day 21 ovulatory progesterone of 92 nmol/L. Day 3 LH was 46.2 IU/L (RR 0.9-9.8 IU/L) and FSH 9.6 IU/L (RR 1.5-10 IU/L), TFTs and prolactin were normal. MRI showed a 2 mm microadenoma within the right side of the pituitary.

An LH day-curve (arranged privately) was analysed by The Doctors Laboratory (Abbott Architect), which showed normal LH concentrations. Concern was raised as to the possibility of heterophilic antibody interference on the Bayer Immuno-1 System (which gave the initial high LH result). To investigate this further, the same sample was analysed on the Roche ElecSys E170 Module, which gave a normal LH result, thus confirming heterophilic antibody interference on the Bayer Immuno-1 System. It was further felt that the microadenoma was an incidental finding and not contributing to the subfertility experienced by this patient.

In conclusion, this patient had high LH concentrations, this led to the differential diagnosis of a gonadotrophin-secreting pituitary adenoma. The LH levels were within normal range on re-testing and this initial result was due to heterophilic antibody interference.

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A carcinoid tumour secreting ACTH localised by PET scan

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A 42 year old man presented to AE with lethargy, mood changes and bilateral swelling of ankles. He had lost weight recently but for the preceding 4 weeks he had been following the Atkins diet in order to reduce his truncal obesity. Investigations revealed a metabolic alkalosis, hypokalaemia, hyperglycaemia, mild hypoalbuminaemia and hypocalcaemia (albumin adjusted). PTH was appropriately elevated and the renin-aldosterone axis was suppressed. Cortisol was 1477 nmol/L and 1742 nmol/L in samples taken at midnight and 08.30 respectively. ACTH was 292 ng/L in the 08.30 sample. Cortisol and ACTH did not suppress overnight after 1 mg dexamethasone. The patient was started on metyrapone to suppress cortisol levels with partial success. Further investigations were undertaken to localise the source of ACTH production.

A CT scan of the adrenals revealed bilateral hypertrophy. An MRI of the pituitary fossa showed no

abnormality. Repeat CT scans of the chest, abdomen and pelvis with contrast were normal. Inferior petrosal sinus sampling showed no evidence for pituitary secretion of ACTH. An octreotide scan was normal. Finally a PET scan was performed which revealed a hot spot at the right heart border. A gated MRI scan confirmed the presence of a lesion adjacent to the right atrium.

A median sternotomy was performed and an anterior mediastinal mass was excised. Histology showed the lesion to be a benign carcinoid tumour. Immunostaining showed focal expression of ACTH. Post operatively cortisol and ACTH levels returned to normal, all the other metabolic abnormalities resolved and the patient made a full recovery.

45

A case of congenital adrenal hyperplasia presenting as bilateral adrenal masses in an adult male

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A 63 year old man was admitted complaining of chest pain, with T wave inversion on his ECG. He was hypertensive with a serum sodium of 130 mmol/L and a spot urine sodium of 77 mmol/L. Urine catecholamines were normal, plasma aldosterone, 500 ng/L (ref: 12-150) and plasma renin 160 mU/L (ref: 5-47) were elevated.

An abdominal CT for suspected aortic aneurysm showed bilateral adrenal masses that were initially considered to be tumours. A short synacthen test gave an impaired cortisol response (254 nmol/L at 30 minutes). 17-hydroxyprogesterone was 889 nmol/L (Ref: 0.9-9.6) and a urine steroid profile confirmed a diagnosis of congenital adrenal hyperplasia (CAH) due to a 21-hydroxylase deficiency. After treatment with hydrocortisone the patient became tired and bloated with increasing weight. Secondary hypogonadism was suggested with a testosterone of 7.7 nmol/L and LH, FSH levels both <0.1 IU/L with impaired responses to LHRH.

A pituitary MRI showed a flattened pituitary gland most likely due to an empty sella. Ultrasound scans of the testes showed multiple high reflectivity masses typical of adrenal rest cells, which are found in poorly controlled CAH patients. Continuing hydrocortisone treatment resulted in primary hypogonadism that required testosterone replacement.

His previous medical history revealed general lassitude, made worse by minor episodes of illness. He remembered being well developed and taller than his peers in childhood, but stopped growing earlier, reaching a height of 1.57 metres.

CAH presenting as bilateral adrenal hyperplasia in a man of this age is most unusual and was a serendipitous

finding. Although pituitary dysfunction was also considered at one stage, treatment with hydrocortisone eventually unmasked primary hypogonadism.

46 An extreme electrolyte disturbance in an otherwise asymptomatic patient in primary care

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A 30 year old lady presented to her GP with a two-month history of blistered hands. A two-year history of secondary amenorrhoea was elicited during this consultation. The GP undertook routine blood sampling, which showed profound hypokalaemia (serum potassium 1.6 mmol/L), hyponatraemia (126 mmol/L) and renal impairment (serum creatinine 182 μ mol/L). Following discussion with the GP, a repeat sample was arranged for the following day (serum potassium 1.4 mmol/L) and urgent admission of the patient.

On admission the lady was well, with no evidence of weakness, lethargy or paraesthesiae. She was noted to be thin, with a BMI of 13.9, but denied weight loss, and had no previous medical history of note. She also claimed to be eating and exercising regularly, and flatly denied nausea, vomiting, diarrhoea and laxative or diuretic abuse. ECG showed flattened T waves, but no other significant changes. On admission her serum magnesium was 1.4 mmol/L.

90 mmol KCL (in 3 L normal saline, 5% dextrose) was administered over a 3 hr period under ECG monitoring. Serum potassium levels reached 2.2 mmol/L. The patient subsequently refused further IV access and additional testing other than for serum potassium. 41 hr after admission the patient discharged herself against medical advice, taking with her a short supply of oral potassium supplements.

It was not possible to make a firm diagnosis, however the patient's history and biochemistry results are highly suggestive of chronic laxative abuse. Whilst extreme serum potassium values are often spurious, it is important to identify those that are genuine and may be life threatening. It is unusual to see this degree of electrolyte disturbance in an otherwise asymptomatic patient in primary care.

47 An extreme case of immunochemical IgG overestimation and heavy chain disease

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An 88 year old female was admitted for further investigations with a 3 week history of a lump in her neck.

Post admission examination revealed rubbery nodes in her axillae, inguinal and posterior/anterior neck regions, non-palpable liver and spleen, with no evidence of infection. There was no weight loss and variable appetite. Imaging revealed no hilar or mediastinal lymphadenopathy. Routine biochemistry revealed U/E, glucose, calcium, magnesium, CRP, TFT and IgA all within their respective reference ranges. LFTs (TP = 70 g/L) showed a reduced albumin of 24 g/L and raised AST (38 U/L). The IgM was slightly decreased at 0.44 g/L and an IgG of 99.9 g/L (neat and on dilution) was obtained via immunoturbidimetry (Beckman LX-20). Her Hb and platelets were low at 9.6 g/dL and 119 respectively. Serum electrophoresis revealed a large discrete band in the fast gamma region with a density of 36.7 g/L. Subsequent immunofixation electrophoresis could only identify a discrete band in the gamma lane, with no evidence of a light chain present. Immunotyping of node aspirate reported "suspicious for lymphoma" but unable to establish clonality as no surface kappa or lambda light chains present. The diagnosis reached was gamma heavy chain disease associated with a low grade non-Hodgkins lymphoma, a very rare condition. It appears that the erroneous [IgG] could be due to malignant lymphoma cells secreting fragments or truncated forms of gamma heavy chains. These possess the epitopes recognised by the antisera of the immunoturbidimetric assay, generating the immune lattices responsible for the increase in turbidity measured. Since these fragments / truncated proteins possess small numbers of peptide bonds, they were not overestimated in the rate biuret method (Beckman LX-20) used for the TP determination. This report highlights an extreme case of the overestimation of a paraprotein by an immunochemical method, in a rare case of gamma heavy chain disease.

48 Rare causes of isolated hyperferritinaemia

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With the almost universal use of serum ferritin assays the finding of an isolated raised ferritin is increasingly common. Although largely associated with inflammatory or chronic disease, an elevated serum ferritin concentration indicates excess iron storage. We present two patients with hyperferritinaemia but otherwise normal iron indices and show that genetic analyses may aid the investigation of hyperferritinaemia and provide a definitive diagnosis.

A 58 year-old man with a history of coeliac disease developed abdominal symptoms. Routine biochemical determinations were normal - apart from a serum ferritin of 1300 μ g/L; this remained elevated for five years

(1575±200 µg/L) with normal ESR and CRP measurements. Liver biopsy was normal and the *HFE* gene was wild-type at C282, and H63. Three years previously, bilateral cataracts had been removed and in the light of this clinical information the *L-Ferritin* gene was sequenced. A heterozygous non-coding point mutation (a6g, Paris1) in the iron-response element of the gene was found. Mutations at this location are responsible for Hereditary Hyperferritinaemic-Cataract Syndrome in which unregulated production of L-ferritin subunits causes early onset of ferruginous l cataracts but no other abnormalities.

The second case is a 46 year-old man with a long history of diarrhoea due to distal ulcerative colitis. Routine biochemistry results were unremarkable apart from a serum ferritin level of 3000 µg/L, with normal CRP and ESR levels. Hepatic biopsy showed iron overload with atypical distribution of dense iron deposits within the Kupffer cells and portal macrophages with light accumulation in hepatocytes. Molecular analysis showed no *HFE* mutation but a novel point mutation in the *Ferroportin* gene (g473t, W158C). Mutations within this gene have recently been described as a rare cause of autosomal dominant haemochromatosis. The phenotype is a consistently elevated serum ferritin level with otherwise normal iron indices but as yet the long-term outcome is uncertain.

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Exonic deletions in an 8-year-old boy with erythropoietic protoporphyria

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Bayer Award

Erythropoietic protoporphyria (EPP) normally presents in childhood with cutaneous photosensitivity caused by the build up of protoporphyrin in the skin as a result of deficient ferrochelatase activity. Evidence suggests that clinical expression of classical EPP requires the coinheritance of a severe ferrochelatase mutation, in trans to the commonly occurring polymorphism, IVS3-48C, which is associated with low enzyme activity.

An 8-year-old boy presented with photosensitivity. A diagnosis of EPP was made following demonstration of increased concentrations of protoporphyrin in erythrocytes and plasma. A referral was made for mutation detection with a view to genetic counselling.

All eleven exons and flanking intronic regions of the ferrochelatase gene were analysed by bi-directional sequencing but no mutation was identified. Samples from the proband and parents were analysed for the IVS3-48C low expression polymorphism. The haplotypes

of the parents were inconsistent with the proband's sample, suggesting the inheritance of a partial gene deletion or non-paternity.

Deletion studies were undertaken using gene dosage analysis. Exons 2-11 of the ferrochelatase gene were amplified by PCR in a multiplex reaction, with incorporation of a fluorescent label and subsequent analysis by gene scanning. Gene dosage was determined by comparison with internal controls. Deletion of exons 3 and 4 in the father and the proband was identified by this method. These results explain the clinical expression of EPP in the proband who, in addition to the deletion, carries the low expression polymorphism. This is in contrast to the father who, although carrying the deletion, is unaffected due to the absence of the low expression polymorphism.

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A fatal case of citrullinaemia

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The urea cycle is the major route for waste nitrogen disposal. It has several steps, each catalysed by a different enzyme. Citrullinaemia, due to a deficiency of arginosuccinate synthase is inherited as an autosomal recessive condition with an incidence of about 1 in 200,000 and both sexes are equally affected. It mostly presents in the neonatal period and is characterised by an accumulation of ammonia.

We present an interesting case of a male infant born at term by vaginal delivery at our hospital, the first child of non-consanguineous Turkish parents. He was discharged when less than 12 hours of age but presented to Casualty at 36 hours of age with poor feeding, reduced sucking and vomiting. On examination he was found to be jittery and to have a plasma glucose of 2.1 mmol/L. He was admitted to the Special Care Baby Unit where he had a generalised seizure and was noted to be hypertonic, hyperreflexic and hypertensive. 10% dextrose and anticonvulsant therapy were commenced and he needed ventilation. His plasma ammonia was 1179 µmol/L, plasma calcium 1.6 mmol/L and plasma lactate 5.6 mmol/L. He was transferred to a tertiary centre where a number of investigations were performed on blood and an ultrasound of the liver was also requested. An infusion of arginine, sodium phenylbutyrate and sodium benzoate was commenced but despite that his plasma ammonia increased to 1615 µmol/L. He gradually became hypotensive and was started on an ionotrope infusion. Subsequently, he had a cardiorespiratory arrest and needed cardiac massage and various

drugs to revive him. His plasma potassium was 6.4 mmol/L and he became oliguric and haemofiltration was commenced. A diagnosis of citrullinaemia was confirmed. His plasma potassium rose to 7.9 mmol/L and plasma ammonia to 3105 $\mu\text{mol/L}$. He arrested on the fourth day of life and died peacefully.

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Severe hypocalcaemia in a patient with tetanus

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A 40 year old intravenous drug user was admitted with cellulitis in his left arm after self injecting. Over a two day period his jaw became painful. By day three he was unable to open his jaw. Tetanus was diagnosed and tetanus immunoglobulin administered. The patient was admitted to intensive care. He was given a tetanus vaccine and was started on atracurium and midazolam. Up to 96 mmol magnesium per day was infused at a rate of 5 mL/hour in an attempt to control his seizures. His serum magnesium levels were monitored aiming to keep levels between 2-4 mmol/L. Serum magnesium levels rose as high as 3.81 mmol/L, corrected calcium levels dropped to as low as 1.40 mmol/L. There was an inverse relationship between magnesium and calcium. 10 mLs of 10% calcium gluconate was administered regularly in an attempt to raise the calcium. Magnesium sulphate continued to be administered. Parathyroid hormone was 3.0 pmol/L with a corrected calcium of 1.52 mmol/L. The patient suffered a generalized tonic-clonic convulsion followed by an asystolic arrest. A troponin T at 12 hours was 0.10 $\mu\text{g/L}$ confirming cardiac damage. Cessation of magnesium infusion enabled normalization of plasma calcium levels. High dose magnesium therapy is an established therapy for eclampsia and premature labour and has been advocated for the control of spasms in tetanus. In eclampsia and premature labour there is evidence that acute hypermagnesaemia is generally tolerated well if levels of 2-4 mmol/L are achieved. There is no evidence for the use of this range in tetanus. Hypermagnesaemia-induced hypocalcaemia has been reported in some women treated for eclampsia with magnesium. Excess magnesium can suppress parathyroid hormone secretion by binding to an extracellular calcium receptor expressed by parathyroid cells resulting in hypocalcaemia. This is the most probable cause of hypocalcaemia in our patient and may have caused his cardiac arrest.

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Hypercalcaemia: an epic tale

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A 74-year-old man with Waldenstrom's macroglobulinaemia, with an IgM-kappa gammopathy (serum IgM 31.50 g/L), was referred to the urologists with prostatic symptoms. His PSA was found to be raised at 17.1 $\mu\text{g/L}$ (0.1-6.5) but quadrant prostate biopsies showed normal histology. During concurrent follow-up in the oncology clinic he was found to have asymptomatic hypercalcaemia (corrected serum calcium of 3.03 mmol/L), which together with a raised PSA prompted a bone scan which was normal. His serum PTH was inappropriately high at 6.30 pmol/L (0.76-7.42). This in the presence of marked hypercalcaemia was consistent with primary hyperparathyroidism. An ultrasound of the neck suggested a parathyroid adenoma.

On review in the Metabolic Clinic, the possibility of spurious hypercalcaemia and *in vitro* paraprotein interference in the PSA assay was investigated. A repeat, uncuffed corrected serum calcium was 3.33 mmol/L, but a simultaneously measured serum ionised calcium was 1.13 mmol/L (1.13-1.32 mmol/L). Serum PSA concentrations were linear on dilution, virtually excluding an *in vitro* paraprotein assay interference. Using a different assay, the respective serum total and free PSA were 52.0 $\mu\text{g/L}$ and 4.20 $\mu\text{g/L}$ (0-4) with a free to total PSA ratio of 8%, these were highly suggestive of prostate malignancy. Further prostatic biopsies confirmed adenocarcinoma.

Results were consistent with spurious hypercalcaemia due the binding of calcium to the IgM paraprotein. Although genuine and spurious hypercalcaemia are well-recognised in some paraproteinaemias such as multiple myeloma, both are vanishingly rare in Waldenstrom's macroglobulinaemia. It is important to identify spurious hypercalcaemia to avoid unnecessary investigations, erroneous diagnosis and inappropriate treatment.

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Hypercalcaemia secondary to sub-cutaneous fat necrosis

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A 4 week old boy was referred by the health visitor with failure to thrive and poor weight gain. Although born at term (wt 4.4 kg), he was delivered in the ambulance en route to hospital and had suffered meconium aspiration, required oxygen and was hypothermic. Over the first 48

hours he was noted to be floppy, hypoglycaemic and had two episodes of seizures. He made an uneventful recovery and was discharged at 8 days.

At review, he appeared clinically well and vital functions were normal. Physical examination showed tender sub-cutaneous lesions over both shoulders, chest and upper thigh. He was admitted for observation with provisional diagnosis of sub-cutaneous fat necrosis (SCFN).

Initial biochemistry showed Na 137 mmol/L, K 4.3 mmol/L, urea 7.9 mmol/L, creatinine 93 μ mol/L. Calcium (uncorrected) was 5.1 mmol/L, with normal albumin and phosphate. LFTs and FBC were unremarkable. Follow-up tests showed PTH <0.8 pmol/L, Vitamin D 22.5 ng/mL (8-15) and urine calcium/creatinine ratio 5.27 (<0.75). X-ray showed no evidence of soft tissue calcification, but nephrocalcinosis was evident on renal ultrasound. Following aggressive treatment with fluids, frusemide, low calcium feeds and phosphate supplements, the serum calcium fell to normal levels within two weeks. Urine calcium fell accordingly. At 6 months, the child is well and developing normally. The skin lesions have disappeared, although the nephrocalcinosis remains. Serum and urine calcium continue to be monitored and diet adjusted accordingly.

SCFN, a neonatal panniculitis of uncertain aetiology, is an uncommon condition, but appears to be associated with birth trauma. Diagnosis is essentially clinical and the characteristic lesions resolve with time. Of importance to the laboratory are the associated complications of hypercalcaemia, hypertriglyceridaemia, thrombocytopenia and hypoglycaemia, which may prove life threatening if not recognised and treated promptly.

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Prospective study of troponin T and troponin I in haemodialysis patients: preliminary report

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Measurement of serum troponin is currently the preferred biochemical test for the diagnosis of MI and for the risk stratification and treatment of ACS. Published data however show that TnT and TnI are elevated in patients with CRF undergoing haemodialysis (HD), even in the absence of apparent cardiac damage, making interpretation difficult. We report preliminary (4 month) data from a year long study, in which both TnT and TnI are being measured in a group of HD patients, without known cardiac damage. The monthly troponin values, inter and intra individual variation, are being recorded and used to formulate guidelines within our Trust for the investigation of MI in patients with CRF.

Two methods with proven track record have been used for this study. Troponin T was measured using a Roche

E170 (Cardiac T) and TnI using a Beckman Coulter Access (AccuTnI). Surplus serum, collected as part of routine pre-dialysis monitoring, was used for both assays. Analysis was carried out as soon as practicable after completion of routine biochemical tests (usually within 48 hours for both markers).

Of 22 patients studied, 12 had complete data for at least 3 months. Of these, 5 would have been reported with negative TnT (<0.03 μ g/L) and 9 with negative TnI (<0.04 μ g/L). The remaining patients had troponin values consistent with some degree of cardiac damage (Range of mean TnT 0.04-0.44 μ g/L, range of mean TnI 0.05-0.14 μ g/L). There was no correlation between TnT and TnI. However, for individual patients the monthly values remained remarkably constant (majority <0.02 of mean) for either marker.

Troponin values in HD patients are variable and interpretation may depend on whether TnT or TnI is measured. Troponin values vary significantly between patients but appear relatively stable within a given individual. Knowledge of this 'baseline' troponin may be helpful if the patient is subsequently suspected of having an acute cardiac event.

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Troponin T levels in stable haemodialysis patients

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Troponin T (TrT) levels are recognised to be elevated in patients with both acute and chronic renal failure (particularly those undergoing haemodialysis). The aim of this study was to measure serum troponin T concentrations in maintenance haemodialysis (HD) patients, pre and post-dialysis.

Seventy-five HD patients (45 male, 30 females, mean age 62 yrs, range 14-87 yrs, mean dialysis duration 2.8 yrs, range 3 months - 13 yrs) with a variety of underlying diagnoses, had venous blood sampled for TrT analysis (Roche E170), pre and post-dialysis. Samples were repeated at the next HD session. Our laboratory currently quotes TrT levels 0.03-0.1 ng/mL as "minimal myocardial necrosis" and >0.1 ng/mL as "consistent with myocardial damage".

TrT levels rose from 0.023 ng/mL (median), (range 0-0.39) to 0.041 ng/mL (range 0-0.47) during the first HD session ($p \leq 0.001$). This was repeated during the second HD session with TrT rising from 0.022 ng/mL (range 0-0.4) to 0.033 ng/mL (range 0-0.51) ($p \leq 0.001$). 47% of HD patients had TrT levels > 0.03 ng/mL and 17% had levels >0.1 ng/mL (consistent with myocardial damage). TrT concentrations were higher in males ($p \leq 0.01$) and increased with age ($p \leq 0.01$). Levels were higher in those with diabetic and ischaemic nephropathy.

In conclusion, this study demonstrates significant elevations in TrT levels in HD patients. TrT concentrations increase significantly post-dialysis. The significance of this is unclear, but may represent underlying coronary artery disease and perhaps there is further myocardial damage during the HD process. Another possible explanation may relate to changes in intravascular volume and fluid balance during the HD process. It is clearly important that these patients are not automatically labelled as suffering myocardial infarction.

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Hyperhomocysteinaemia in haemodialysis patients

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Plasma total homocysteine (tHcy) is frequently elevated in patients with end stage renal failure. Elevated blood homocysteine appears to be associated with an increased risk of cardiovascular morbidity and mortality, a major cause of death, among these individuals. The cause of hyperhomocysteinemia in renal failure is complex and not well understood. High-efficiency hemodialysis may induce deficiency of hydrosoluble vitamins. Our aim was to analyze the influence of uraemia and haemodialysis on the levels of tHcy, folate (Fol) and Vitamin B12 (B12). We investigate 30 chronic dialysis patients (DPG) not supplemented with hydrosoluble vitamins. As a control group (CG) we used 70 age- and sex-matched subjects. Serum tHcy was determined using Abbott IMX FP-assay. Fol and B12 were determined using Bayer ACS:180 assays. We found significantly higher levels of tHcy in DPG compared to CG ($36.62 \pm 10.1 \mu\text{mol/L}$ vs. $13.89 \pm 5.94 \mu\text{mol/L}$ $p < 0.001$); very low Fol ($3.44 \pm 3.14 \text{ nmol/L}$ vs. $20.13 \pm 11.53 \text{ nmol/L}$ $p < 0.001$) and lower B12 ($240.3 \pm 132 \text{ pmol/L}$ vs. $310.8 \pm 131.12 \text{ pmol/L}$ $p < 0.001$).

Hyperhomocysteinaemia was found in 100% of our unsupplemented haemodialysis patients; high Hcy positively correlated with creatininaemia ($r = 0.274$, $p = 0.142$). Nearly 100% of our unsupplemented hemodialysis patients were deficient in folic acid and B12. This may constitute a risk for severe vascular damage in dialysis patients.

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Association of serum erythropoietin with anaemia and nephropathy in patients with type 2 diabetes

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Anaemia is an important component of diabetic nephropathy which is a common cause of end-stage

renal disease. We postulate that inappropriately low erythropoietin concentration occurs early in the development of nephropathy even in patients without anaemia. This study investigates the relationships between the degree of nephropathy and erythropoietin concentration in patients with type 2 diabetes.

We measured serum erythropoietin (Epo), ferritin and complete blood count in 161 type 2 diabetic patients (93 females and 68 males, mean (\pm SD) age: $58.85 (\pm 10.76)$, diabetes duration: $12.3 (\pm 8.14)$ yrs. Serum creatinine and calculated creatinine clearance (Cockcroft-Gault) were used as markers of glomerular filtration rate and urine albumin:creatinine ratio was determined to classify patients as normo-, micro- or macro-albuminuric.

Normal Epo = 5-36 mU/mL. The mean (95% confidence interval, CI) Epo in patients with normo-, micro- and macroalbuminuria were 7.96 (6.12-9.81) mU/mL; 5.02 (3.74-6.30) and 4.29 (2.35-6.24) mU/mL respectively. Ferritin showed the opposite trend with mean (CI) values of 100.71 (83.54-117.87) ng/mL; 110.58 (80.23-140.92) ng/mL and 161.92 (56.90-266.94) ng/mL in patients with normo-, micro- and macro-albuminuria respectively. Mean Hb was not significantly different between patients with normo- (138 g/L); micro- (136 g/L) and macro-albuminuria (131 g/L) ($p = 0.46$). Spearman correlation analyses showed that Epo was significantly correlated with serum creatinine ($r = -0.16$; $p = 0.48$); albumin:creatinine ratio ($r = -0.18$; $p = 0.04$); Hb ($r = 0.23$; $p = 0.004$). However partial correlation analyses correcting for age and sex showed that Epo correlated with Hb ($p < 0.0001$); MCV ($p < 0.001$) and creatinine clearance ($p < 0.001$) only. There was no correlation with age, duration of diabetes and HbA1c.

Epo concentration is inappropriately low in diabetic patients independently of the ferritin and haemoglobin concentrations. There is need to determine serum Epo as low erythropoietin may precede the onset of incipient nephropathy in diabetic subjects.

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Changes in renal protein selectivity during abdominal aortic surgery: the effect of different intravenous colloids

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Microalbuminuria following insults such as trauma or surgery is predictive of outcome, and the type of intravenous colloid used influences the degree of proteinuria. This study aimed to determine whether low-level proteinuria associated with surgery is related to changes in glomerular selectivity and the effect of different intravenous colloids.

Sixty patients were randomised to receive either 6% hydroxyethyl Starch (HES) with a mean Mwt 200 kDa, 6% HES mean Mwt 130 kDa or 4% gelatine mean Mwt 30kDa. Urine albumin, IgG and alpha 1 microglobulin (A1M) were measured by automated immunoturbidimetry and expressed as the protein/creatinine ratio, and plasma albumin and IgG were measured to calculate urine protein selectivity.

For all sixty patients median (95% CI) urine albumin rose from 2.6 (1.9-8.3) pre op to 23.0 (11.7-33.3) mg/mmoL following clamp release ($p < 0.0001$) and urine IgG rose from 0.6 (0.5-1.3) to 5.0 (3.2-8.8) mg/mmoL respectively ($p = 0.001$). For all patients median A1M rose from 1.3 (0.8-1.5) pre op to a maximum of 5.9 (5.2-7.5) mg/mmoL 48 hours post operatively ($p < 0.0001$). Compared with the gelatine group, both HES 200 and HES 130 groups showed lower A1M levels at 2, 4, 6, 8 and 12 hours post operatively ($p < 0.02$). There was no change in protein selectivity during surgery or post operatively, however compared with the gelatine groups, the HES 130 group showed higher protein selectivities at 2, 4, 8 and 12 hours post operatively (ANOVA < 0.01), and the HES 200 group showed higher selectivity at 6 hours postoperatively ($p = 0.03$).

Proteinuria associated with AAA surgery is glomerular rather than tubular in origin and is not associated with a change in protein selectivity. Post operative proteinuria is predominantly tubular in origin and is influenced by the type of colloid used, being most pronounced in patients given gelatine. Patients given gelatine also showed lower protein selectivity compared with both HES groups, particularly HES 130. These data suggest compared with gelatine, HES is renal protective.

59 Urinary calcium/creatinine ratio in healthy young children of North Israel

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Idiopathic hypercalciuria is one of the most common mineral disorders causing urolithiasis and nephrolithiasis in children. Reference values for normal calcium excretion in children are important to identify abnormal patterns of excretion for early detection of hypercalciuric states. Calcium excretion traditionally has been evaluated by assessing 24 h urine samples which are difficult to collect in children. The usefulness of measuring urine calcium excretion in relation to creatinine excretion in random urine samples has been demonstrated in adults. Studies in several populations of children of diverse ethnicity and calcium intake have revealed rather different normal values for calcium/creatinine (Ca/Cr) ratios. These group studies often lacked standardization of

urine collection, time of the day and the fasting state of the children prior to the test. However, many studies confirm a decrease in the Ca/Cr ratio with age; from the age of 6 years the ratio becomes stabilized around the adult value.

Our objective was to determine reference values for urinary Ca/Cr ratio in healthy children up to 6 years of age from northern Israel

The study was performed on 122 healthy children, aged 1 month to 6 years. First morning urine samples were taken after overnight fasting (whenever possible); samples were acidified before analysis. Calcium and creatinine levels were measured on either Hitachi 911 or Olympus 2700 analyzers. The study was performed in cooperation with the nephrology unit of Rambam Medical Center (with local Helsinki Committee approval).

The two methods differ significantly. The age was not significant as an explanatory variable for the measurements. The percentiles (0.025, 0.975) were evaluated based on the normality assumption using the simple mean and standard deviations of log Ca/Cr. The 97.5th percentile for Ca/Cr on Hitachi 911 and Olympus 2700 was 0.57 (mg/mg) and 0.62(mg/mg), respectively.

We provide reference values for urinary Ca/Cr for children younger than 6 years, which can help pediatricians screen for hypercalciuria.

60 Urine collection pads: are samples reliable for routine urine biochemical investigations?

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Urine collection pads (UCP) are widely used for microbiological examinations as an easy, non-invasive collection method for young children still in nappies. The technique overcomes many of the disadvantages of other urine collection methods e.g. clean catch, bag, catheterisation etc.

We wanted to extend the UCP technique for clinical use in routine biochemical analyses. Other work has shown problems when using nappy materials for urine collection.

Urine samples from paediatric and adult volunteers were examined prior to and after incubation at 37°C on UCP (Newcastle Urine Collection Pads, Ontex Ltd., NHS Supplies) (to simulate the environment of a pad enclosed in a child's nappy) for the following analytes:

Sodium, potassium, chloride, urea, creatinine, calcium, phosphate, urate, protein (Olympus AU6000 methodology) osmolality (freezing point depression) and semi-quantitative stick testing.

The study has demonstrated that use of UCPs for urine collection gives clinically reliable results for a wide range of biochemical analytes. For urine protein a reduction (mean 10.5%) from the UCP is evident, however it is not

sufficiently large to affect diagnosis or clinical decision making in the context of nephritis/childhood nephrotic syndrome.

UCPs provide a convenient and reliable technique for the collection of urine samples for routine biochemical analysis.

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Guidelines for performance of the sweat test: highlighting a clinical governance problem?

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Draft guidelines (2001) recommend that the Orion electrode should not be used to analyse sweat *in situ* immediately after stimulation. In our hospital the Orion probe was used until April 2001. It was practice to refer all positive Orion probe results to the Regional CF clinic for a repeat sweat test using the Wescor collection system, and analysis of sodium and chloride.

We reviewed all cases from January 2000 to April 2001 to decide if the tests needed repeating. If there was sufficient concern, families were contacted by letter. According to the degree of concern, they were offered a clinic appointment or a repeat sweat test (using the Wescor collection system and analysis of sodium and chloride). All GPs in the area were informed of the plan in writing.

There were 105 sweat tests performed on 100 patients. 26 patients had seen a local expert or had a negative sweat test performed at another hospital.

Thirty-eight patients were invited in writing for a repeat sweat test, and were contacted by telephone the day before the test. 28 of these failed to attend, of which 20 did not answer the telephone or were disconnected.

Thirty-seven patients were written to and invited to arrange a clinic appointment. Two families requested appointments but neither attended.

All results that were repeated were normal.

There was very little success in contacting families despite reasonable efforts. There was little interest in the offer of a repeat test. This information may be helpful to hospitals considering whether to recall patients following the new guidelines.

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Management outcomes when using two different POCT methods for neonatal bilirubin: are we using appropriate action limits?

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Hyperbilirubinaemia is a common finding in neonates and the immediate treatment is phototherapy to promote

bilirubin excretion. In more serious cases, exchange transfusion is indicated. The decision to treat involves the use of action limit curves, based on age and total serum bilirubin. It is necessary to use action limits appropriate to the instrument used for bilirubin measurement.

The Special Care baby unit at Queen Elizabeth Hospital currently uses both a Pfaff Bilirubinometer and an ABL 700 Blood-Gas analyzer (Radiometer) for the measurement of total bilirubin in jaundiced neonates.

We have shown that the relationship between the Pfaff Bilirubinometer (y) and the ABL 700 (x) to be $y = 0.91x + 49$ for measurements of total bilirubin on jaundiced neonates. The action limits currently used by our Special Care Baby unit may be inappropriate because we believe these were derived using a Bilirubinometer. This bias of 10% may therefore lead to under-treatment of jaundiced neonates if the ABL 700 is used, increasing the risk of kernicterus. Our aim is to produce appropriate action limit curves for the ABL 700 Blood-Gas analyzer.

The reason behind the difference is unclear; both instruments use a similar spectrophotometric-based method. The probable cause is a matrix effect since the ABL 700 uses whole blood and bilirubinometers use serum samples.

Depending on the instrument used, the neonate may be under-treated. Conversely, the baby may spend unnecessary time in hospital receiving inappropriate phototherapy or blood exchange transfusion. This is an issue of clinical governance to be considered by both medical and scientific staff. We recommend that Biochemistry departments investigate the POCT instruments used for the measurement of total bilirubin since the action limit curves used by clinical staff may be unsuitable.

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Evaluation of a new ammonia meter: Pocketchem BA®

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Measurement of ammonia is accepted as one of the first line tests in the investigation of suspected inherited metabolic disease (IMD) in the acutely ill infant or child. To facilitate the rapid screening of such infants for hyperammonaemia, blood ammonia meters using dry strip chemistry and reflectance spectrometry have been widely used particularly in secondary health care facilities. However, because of clinical incidents and poor performance in the WEQAS External Quality Assessment (EQA) scheme for ammonia, the British Inherited Metabolic Disease Group was obliged to notify the Medical Devices Agency.

A new model of ammonia meter - Pocketchem BA® - has been designed and is in receipt of a CE mark. The meter includes a number of features to comply with point of care guidelines and to prevent the recurrence of similar incidents.

An evaluation of the new meter was conducted at a District General Hospital according to NCCLS guidelines. The performance of the instrument was comparable to the predecessor - the BAC II - with precision of 3.5% for viscosity adjusted IQC of 100 mmoles/L and recovery for added ammonium chloride up to 200 mmoles/L of between 97-127%. Samples with ammonia concentration exceeding 286 mmoles/L registered as out of range "Hi". The analytical range can be extended up to 500 mmoles/L using a viscosity adjusted diluent. There was a proportional bias of 10% in comparing whole blood and plasma ammonia (measured using an enzymatic method) concentrations. The PocketChem BA™® like its predecessor is suitable for use in clinical biochemistry laboratories as long as the device is properly commissioned and the standard operating procedures document the limitations of the device and the analytical precautions. Like all laboratory tests which are infrequently requested, periodic retraining and revalidation of staff is required.

64 Paediatric biochemistry training in the UK: response to a questionnaire circulated by the ACB Trainees Committee 2003

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The ACB Trainees committee circulated a questionnaire to trainees to establish the extent of training and experience in paediatric biochemistry.

The ACB database was used to select members who joined the profession after 1993 and those studying for the MRCPPath. examination. 86% (234/273) of those selected responded to the questionnaire, with 19% (45/234) excluding themselves.

140 responders entered the profession via the Grade A training scheme. 71% of Grade A trainees spent between 1 week and 9 months at a paediatric hospital and 62% visited a neonatal screening department. 20% received no formal paediatric tuition and 26% did not attend any relevant meetings.

29 replies were from biochemists in higher specialist training posts. 8/29 gained experience of paediatric biochemistry with 3/8 responders in specialist paediatric posts. 52% had attended meetings relating to paediatrics.

120 replies were from permanent Grade B8-24 biochemists. 21% had spent time in a paediatric unit whilst in a Grade B post. However, 76% of these were employed

in paediatric departments. 67% had attended relevant meetings.

46% responders felt training in paediatric biochemistry was inadequate. When asked to rank training in genetics, haematology, paediatrics and toxicology the majority of trainees considered paediatric biochemistry to be most important. Less than 50% of responders were aware of BIMDG, SSIEM, ERNDIM or the paediatric biochemistry network. Each of these organisations has a role in paediatric biochemistry education. A summary of the results with information about these various organisations will be sent to those who completed the questionnaire to improve awareness of training resources.

This questionnaire has evaluated the current level of paediatric biochemistry training and provides valuable information identifying where training is being given and highlights areas for improvement. In addition, this information has been used to help gain Department of Health funding for paediatric biochemistry training.

65 Replacement of immunoassay by LC/MS/MS for the routine measurement of drugs of abuse in oral fluid

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The measurement of drugs of abuse in oral fluid samples provides better sample integrity removing the problems of adulteration or substitution which may occur with urine collections. However, the concentrations of drugs or their metabolites in oral fluid are much lower than those found in urine and confirmation of a particular drug requires analysis using sensitive GC/MS methods. The introduction of LC/MS/MS allows individual drugs or their metabolites to be measured in oral fluid samples at the cut-off concentrations used in immunoassays and at reduced cost compared to commercial immunoassays.

A method for measuring drugs of abuse in oral fluid samples was developed using a Shimadzu SIL-HT autosampler and binary pump system linked to an Applied Biosystem API 3000 tandem mass spectrometer. Oral fluid samples were collected using the Orasure device. Samples were spiked with deuterated morphine, cocaine, methadone and diazepam and diluted with an alkaline buffer. The drugs were extracted using diethyl ether. Solvent was removed using nitrogen and the residues reconstituted in mobile phase (25 mM ammonium acetate, 5% methanol in water). Gradient elution was carried out on a 50 x 3 mm Hypersil C8 column using methanol (containing 0.05% formic acid and 2% isopropanol) as a second mobile phase for a run time of 6.5 minutes. This method allowed the identification of morphine, codeine and dihydrocodeine above a cut-off

concentration of 10 ng/mL, 6-monoacetylmorphine above 1 ng/mL, cocaine above 1 ng/mL and methadone above 5 ng/mL in samples from patients attending a drug treatment clinic. The benzodiazepines, diazepam, nordiazepam, oxazepam, temazepam, nitrazepam and 7-aminonitrazepam could be detected in patient samples above a cut-off concentration of 1 ng/mL. Results from the LC/MS/MS gave good agreement with the current immunoassay.

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A comparison of GC-MS, immunoassay and liquid-liquid extraction with HPTLC in the detection of cannabis in routine urine drug screens

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Cannabis abuse is detected through identification of the major urinary metabolite delta-9-tetrahydrocannabinol-9-carboxylic acid (delta-9-THC-COOH). Traditionally, our laboratory has utilised liquid-liquid extraction and HPTLC, however this is labour intensive and interpretation is subjective. The use of immunoassay as a screening method is well documented but positives must be confirmed owing to the incidence of false positive results. The aim of the study was to compare immunoassay to HPTLC and compare the performance of a GC-MS method to immunoassay for detection of delta-9-THC-COOH.

A total of 388 urine samples (10 mL) positive for delta-9-THC-COOH at cut off >50 ng/mL by immunoassay (Roche) were hydrolysed (11.8 M KOH), incubated (57°C 10 min) and maleic acid (4 mL 2M) added. delta-9-THC-COOH was extracted in hexane:ethylacetate (9:1). Dried extracts were reconstituted in 100 mL dichloromethane:propan-2-ol (3:1) and spotted onto HPTLC plates. delta-9-THC-COOH was detected as a red spot after dipping plates in a solution of Fast Blue BB salt in dichloromethane and immersing in an atmosphere of diethylamine vapours. The GC-MS method was adapted from the above (1 mL urine, 0.1 mL 11.8 M KOH) with addition of deuterated delta-9-THC-COOH d3 as internal standard. Dried extracts were silylated using MSTFA, heated at 60°C for 20 minutes and transferred to GC vials.

Immunoassay false positive rate at cut off >50 mg/mL was 5.9%. Values for each HPTLC positive showed overlap and ranged between 51.7- >1000 ng/mL (weak positive), 51.2-933.1 ng/mL (positive) and 71.2 - >1000 ng/mL (strong positive). Correlation between GC-MS and immunoassay (n=226) was $r=0.74$ with immunoassay showing a 76.0% positive bias compared to GCMS. The immunoassay cut off of 50 ng/mL equated to a cut-off

of 20 ng/mL using GC-MS. No false positives were observed.

In conclusion, the GC-MS method is rapid and more specific than our previous two methods and has been adopted for routine confirmation of immunoassay positive urine samples.

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Ranitidine and false positive amphetamine results on the Roche Integra

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Pressures to reduce the cost and turnaround time of analytical testing make it tempting to discontinue routine confirmation of urine specimens positive for drugs of abuse by immunoassay. However, the requirement for confirmation is also driven by the positive predictive value of the screening tests.

In accordance with the UK laboratory guidelines for legally defensible workplace drug testing we use a screen positive cut-off for amphetamines of 300 µg/mL, this is considerably lower than the cut-off of 1000 µg/mL quoted in the Roche literature but is more appropriate in our setting.

We were becoming concerned that the false positive screening rate for amphetamines was becoming unacceptably high. We reviewed the confirmatory rate of urine specimens positive for amphetamines on the Roche Integra from January to December 2003. There were 405 false positive screens for amphetamines during this 12-month period giving a positive predictive value of 49%. We established that the low positive predictive value of the amphetamine assay in our laboratory was primarily due to the use of ranitidine (Zantac). Ranitidine is an extremely commonly prescribed drug and is now available in some over the counter preparations (for example Gavilast).

Finally, due to the sensitivity of the Integra amphetamine assay to ranitidine combined with our requirement for a sensitive screening assay we continue to require confirmation of all our positive amphetamine screens results.

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Analysis of morphine and its principal glucuronide metabolites in post mortem samples

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Morphine is a powerful opiate analgesic drug commonly used for the treatment of chronic pain. It is also the major metabolite of heroin, a drug that is commonly abused for its psychoactive properties. A major route for

the metabolism of morphine is glucuronidation, to the 3- and 6-glucuronide metabolites, the latter of which is a minor metabolite but has been shown to have strong analgesic properties. It has been proposed that this metabolite may have a potential role in the development of toxic symptoms in some opiate fatalities.

The aim of this project was to develop an automated solid phase extraction process, linked to a HPLC system, for the isolation of morphine and its glucuronide metabolites morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Extraction was accomplished using an automated Gilson ASPEC system which is capable of extracting up to twenty samples, unsupervised. The solid phase extraction system was optimised with a particular emphasis on the simultaneous isolation of the polar glucuronide metabolites and the non-polar parent compound morphine. Following extraction, quantitation of morphine and metabolites was achieved using high performance liquid chromatography with fluorescence detection. Again the chromatography system was optimised to allow the simultaneous detection of polar and non-polar compounds.

The optimised analytical procedure uses a single 3ml capacity C18 extraction column and 500 μ L of whole blood or serum for each analyses. Chromatographic separation was achieved using a mobile phase consisting of 2 mM sodium dodecyl sulphate in 0.05% (v/v) phosphoric acid:acetonitrile (72:28 v/v). Fluorescence detection was carried out using an excitation wavelength of 245 nm and an emission wavelength of 335 nm.

The problems associated with the isolation of compounds of interest in post mortem samples is discussed.

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Stated interval since last heroin use and urine drug analysis among clients of a drug misuse centre

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The aim of this study was to find out if clients attending a substance misuse centre who admitted to using heroin were accurate when stating the time of last use.

Clients who had used heroin between visits were asked the dosage time, amount and the route of administration. A urine sample was collected for drug screening by EMIT® and opiate confirmation using a qualitative GCMS method. GCMS results exceeding the cut-off concentrations for morphine (MOR, 1000 μ g/L), 6-monoacetyl morphine (MAM, 10 μ g/L) and heroin (HER, 10 μ g/L) were deemed positive. These were grouped according to the interval between urine collection and the time of last heroin dose as stated by the client.

Last dose information accompanied 387 urine specimens. Heroin doses varied from 1/4 bag to 6 bags (median 1 bag, containing approximately 30 mg heroin base) given intravenously (66%), or smoked (34%). The proportion of urine samples found positive for heroin metabolites declined as the time to last dose increased: 0-12 h: MOR 94%, MAM 91%, HER 65% (n=161); 13-24 h: MOR 59%, MAM 65%, HER 39% (n=130); 25-48 h: MOR 44%, MAM 57%, HER 35% (n=54); 49-72 h: MOR 27%, MAM 50%, HER 14% (n=22); 73-120 h: MOR 30%, MAM 20%, HER 10% (n=20).

Published data show that MAM is cleared from urine within 12 hours of heroin dosage. 58% of our clients claiming 13-120 hours since last heroin use had MAM-positive urine. This group has clearly exaggerated the interval since last heroin use.

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Aluminium mediated toxicity to kidney cells is relatively minor related to lipid peroxidation and cell membrane disruption not apoptosis

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Aluminium toxicity to brain, bone and recently to kidney proximal tubular cells (PTCs) is well described. To explain these effects we have investigated whether lipid peroxidation and/or cellular glutathione (GSH) depletion is involved and the relevance of apoptosis.

Monolayers of PTCs in T25 flasks were exposed to 100 μ mol/L of Al-citrate (test) or cisplatin (positive control) or culture medium alone (negative control) and incubated for 48 hours in standard cell culture conditions on 3 different occasions. The cells (unattached and attached) were collected, washed with cold PBS and freeze/thaw lysed. The cell lysate and culture medium were analysed for malondialdehyde (MDA) using thiobarbituric acid and separation by HPLC and GSH using dithiobis reduction. Separate apoptosis studies were performed using DAPI staining and TUNEL assay.

Aluminium and cisplatin treatment resulted in an increase in cellular MDA (3.4 ± 0.8 and 5.9 ± 2 , respectively against control 1.5 ± 0.2 μ mol/mg protein, n=3, $p < 0.05$); GSH changes were not significant (1.04 ± 0.08 and 0.52 ± 0.26 against control 0.80 ± 0.16 mmol/L, n=3, $p > 0.05$). The MDA and GSH release into the culture media was not different for aluminium (0.86 ± 0.24 , control 0.63 ± 18.93 μ mol/L n=3, $p > 0.05$), but MDA increased with cisplatin (1.59 ± 0.19 μ mol/L, n=3, $p < 0.05$).

Aluminium exposure led to a slight increase in the numbers of apoptotic bodies as assessed by DAPI staining. However, Tunel assay with fluorescent microscopy or flow cytometry showed no evidence of DNA damage

even with 300 $\mu\text{mol/L}$ aluminium. Marked DNA damage was observed with cisplatin treated cells.

This study has indicated some evidence of lipid peroxidation in Al-induced injury to kidney proximal tubular cells. In spite of contradictory reports, our findings on kidney cells showed no evidence of DNA damage. The mechanism of initial Al cell toxicity is most likely related to lipid peroxidation and cell membrane disruption.

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Transplant indication, PCV and serum albumin and creatinine may determine the overestimate of blood tacrolimus levels by immunoassay vs LC-MS/MS

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The immunosuppressant tacrolimus requires monitoring to assist dosage adjustments. LC-MS/MS provides an alternative to the immunoassays in current use and equivalence of results was compared in this study.

Samples were assayed by the Abbott Tacrolimus II microparticle enzyme immunoassay (MEIA) on the IMx and by LC-MS/MS (Keevil *et al.* 2002). Blood samples were divided into 6 groups from in-/out- adult (AL) or paediatric (PL) liver recipients (120/165 and 102/155 samples, respectively), from renal recipients (101), and from bone marrow (BM) recipients (62). PCV, INR, creatinine, bilirubin, AST, ALP, albumin and phosphate results were obtained when available.

LC-MS/MS results were lower than MEIA results by a median (range) of -10.4% (-100.0 to 246.2%) for all samples, but with good agreement (intercept -0.157 [-0.418 to 0.103] and slope 0.921 [0.893 to 0.949]; Deming, MEIA as reference, [95% CL]). The bias (range) for each group was AL in, -23.1% (-99.4 to 96.6%) and out, -5.9% (-100.0 to 91.9%); PL in, -9.5 (-53.2 to 138.6%) and out, -2.2% (-46.8 to 116.7%); renal -11.6% (-100.0 to 246.2%); BM recipients, -25.7% (-100.0 to 75.0%). These differences were significant ($p \leq 0.001$, one-way ANOVA).

Stepwise linear regression analysis showed a significant effect of albumin ($p \leq 0.001$), albumin and PCV (both $p \leq 0.001$) and albumin, PCV and creatinine ($p \leq 0.001$, 0.002 and 0.007 respectively) on the inter-assay bias of all the samples. PCV had a significant effect on the bias ($p \leq 0.001$) in the AL in- and out-patient groups and a weak effect ($p = 0.04$) in the PL out-patient group, but there were no significant effects in PL in-patients. Albumin ($p = 0.02$) and creatinine ($p = 0.01$) had a significant effect on the bias in the BM, but not in the renal group.

These data indicate there is no universal proportionality between tacrolimus levels measured by MEIA and LC-MS/MS. A possible cause is cross-reacting tacrolimus metabolites contributing to an overestimate with the MEIA thus increasing the risk of overestimating drug efficacy, particularly in some liver and bone marrow recipients.

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Instability of serum folate: a problem with both patient and NEQAS samples

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In July 2003 we were informed by the organiser of the UK NEQAS Haematinics scheme that we had unsatisfactory poor performance for serum folate due to an average bias of +30% from target values, Bayer Centaur trimmed group means, for the previous four distributions.

We suspected that degradation of folate in the NEQAS samples contributed to this bias. Our laboratory assays these samples on the day of receipt, usually the day following dispatch, as recommended by the scheme organiser. Other UK participants store samples for several days before analysis and non-UK laboratories, approximately one third of the total, take up to five days to receive NEQAS samples.

Patient and NEQAS samples were analysed on the day of receipt (day 1) and on each of the subsequent three days following storage at room temperature. The day 1 concentrations of the patient samples ranged from 2.1 to 12.7 μL ($n = 12$) and the NEQAS samples from 1.8 to 13.1 $\mu\text{g/L}$ ($n = 12$).

Mean patient values were 91% on day 2, 82% on day 3 and 72% on day 4. Mean NEQAS values were 90% on day 2, 77% on day 3 and 66% on day 4. These mean values were all significantly lower ($p < 0.05$) than those obtained the preceding day.

In the second half of 2003 the scheme organiser began adding sodium ascorbate to the pools used in the scheme. This has improved the between laboratory CV of the Centaur group from 27.0% (July 2003) to 8.4% (October 2003). Our positive bias has fallen during the same period to +1.8%.

In summary, the instability of serum folate at ambient temperatures means that patient and NEQAS samples should be stored at 4°C on receipt and assayed within 24 hours, or stored frozen. Ascorbate improves the stability of serum folate but does not completely prevent its degradation.

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Serum free light chain assays can identify minimal residual disease in multiple myeloma patients who are immunofixation negative

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Oral Presentation

Measurement of serum free light chain (FLC) concentrations has been shown to be more sensitive than electrophoresis for detection and monitoring of nonsecretory multiple myeloma and light chain only multiple myeloma (LCMM). After treatment, however, complete response (CR) is currently defined as an absence of paraprotein in serum and urine by immunofixation electrophoresis (IFE). The aim of this study was to determine whether the serum free kappa/lambda ratio is a more sensitive measure of CR in LCMM and intact immunoglobulin multiple myeloma (IIMM). FLC concentrations were measured in the sera of 85 patients from the MRC VI and VII Myeloma trials who were determined to have achieved CR by IFE. Of the 85, 31 had LCMM and 54 had IIMM. 11/31 LCMM and 37/54 IIMM patients, had normal serum FLC ratios, in agreement with the IFE results. The remaining 20/31 LCMM patients and 17/54 IIMM patients had abnormal FLC ratios indicating the presence of residual disease. Where available (n=44), overall survival (OS) data, indicated that patients who had abnormal FLC ratios, had shorter OS than those who were IFE negative with normal FLC ratios (mean OS of 990 versus 1188 days for LCMM patients and 891 days versus 1430 days for IIMM). A t-test analysis of the data revealed that the differences reached significance for the IIMM patients ($p < 0.03$) but not the LCMM patients. It has previously been shown that serum FLC assays are more sensitive than urine FLC tests for the determination of CR in LCMM and this increased sensitivity is due, in part, to normal renal function preventing light chains entering the urine. The current study indicates that serum FLC assays also identify residual disease in some IFE negative IIMM patients and that abnormal FLC ratios may be predictive of a reduced OS.

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Development of a latex-enhanced rheumatoid factor assay for the Minineph®

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Rheumatoid factors (RF) are naturally occurring IgG, IgA or IgM class antibodies directed against the Fc portion of aggregated IgG molecules. The measurement of RF can be useful diagnostically, e.g. with rheumatoid arthritis the presence of RF indicates a poorer prognosis than in its absence. Elevated RF concentrations are frequently associated with a difficult clinical course and

generalised disease in rheumatoid arthritis. A latex-enhanced RF kit has been developed for use on the Minineph®. The calibration curve was stored on a pre-programmed magnetic swipe card and loaded into the machine's memory. Curve validity was checked by control sample assay. The assay took 2 mins and was read at end-point. The assay range was 30-484 IU/mL using a 1/40 sample dilution, with a sensitivity of 8 IU/mL. Intra- and inter-assay precision was assessed at two antigen levels 237 and 114 IU/mL. The coefficients of variation were for the high sample 2.5% and 5.8% respectively and for the low sample 4.2% and 4.3%. To assess assay linearity, serially diluted serum samples were measured and expected results were compared with actual results. The assay showed a high degree of linearity, when expected values were regressed against measured values $y = 0.97x + 10.9$, $r = 0.999$. No significant interference (within $\pm 8\%$) was observed with haemoglobin (500 mg/dL), bilirubin (20 mg/dL) or lipemic samples (chyle 1670 turbidity units). Comparison was made between this assay (y) and the Kamiya RF K-assay for use on the Roche Hitachi 911 (x) over a range of 11 - 4430 IU/mL: $y = 0.951x + 0.79$ ($r = 0.989$, $n = 42$). Good agreement was demonstrated over the normal and elevated ranges of RF. We conclude that this assay measures RF precisely, accurately and rapidly and may be of use in laboratories with a lower throughput where the expense of a fully automated analyser can not be justified.

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Development and evaluation of IgG subclass assays on the Olympus AU400

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Measurement of IgG subclasses (IgGSc) is useful in the diagnosis of IgG deficiencies where a deficiency in one IgGSc is masked by normal total IgG levels. Assays for measurement of IgGSc in serum have been available for many years and are routinely used in many immunology laboratories. Increasingly, immunoassays are now being performed on multifunctional analyzers that are capable of performing immunological assays as well as chemistry assays. These analyzers have the advantages that they are very precise and can cope with a high sample throughput. Here we describe development of IgGSc assays for use on the Olympus AU400 and evaluate their performance. The assay range for IgG1 was 1500-40000 mg/L, for IgG2 486-13125 mg/L, for IgG3 78-1250 mg/L and for IgG4 20-750 mg/L. Assay time was 8 minutes for all four IgGSc assays. Intra- and inter-run precision was assessed and the coefficient of variation ranged from 1.0-5.5% and 1.2-5.1% respectively.

Interference was within $\pm 5\%$ when bilirubin (300 mg/L), hemoglobin (5 g/L) or chyle (1,930 formazine turbidity units) was added to serum samples with known IgGSc concentrations. Linearity was assessed by assay of serially diluted serum samples and comparison of expected with measured results by regression analysis:- IgG1: $y=0.985x + 416$ mg/L, $r=1.00$; IgG2: $y=0.996x + 29.7$ mg/L, $r=1.00$; IgG3: $y=0.997x + 2.2$ mg/L, $r=1.00$; IgG4: $y=1.01x + 2.4$ mg/L, $r=1.00$. All assays were linear over the range tested. Comparison was made with the TBS IgGSc assays for the Dade Behring BN®. Serum samples from normal subjects ($n=100$) were assayed for IgGSc on both systems and regression analysis of the results shows good agreement:- IgG1: $y=0.98x + 365$ mg/L, $r=0.99$; IgG2: $y=1.04x + 42$ mg/L, $r=0.99$; IgG3: $y=0.94x + 38$ mg/L, $r=0.99$; IgG4: $y=1.05x + 11$ mg/L, $r=0.99$. The IgGSc assays for the Olympus AU400 provide a rapid, precise method of measuring IgGSc in serum and show good agreement with existing assays

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Evaluation of latex-enhanced turbidimetric reagents for measuring IgG subclasses on the Roche Modular P

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Measurement of IgG subclasses (IgGSc) is useful in the diagnosis of IgG deficiencies where a deficiency in one IgGSc is masked by elevated levels of the other IgGSc. Assays for measurement of IgGSc in serum have been available for many years and are routinely used in many immunology laboratories. Increasingly, immunoassays are now being performed on multifunctional analyzers that are capable of performing immunological assays as well as chemistry assays. These analyzers have the advantages that they are very precise and can cope with a high sample throughput. Here we describe development of IgGSc assays for use on the Roche Modular P and evaluate their performance. The assay range for IgG1 was 1000-20000 mg/L, for IgG2 600-10000 mg/L, for IgG3 75-1200 mg/L and for IgG4 68-810 mg/L. Assay time was 10 minutes for all four IgGSc assays. Intra- and inter-run precision was assessed and the coefficient of variation ranged from 0.8-1.7% and 1.1-3.6% respectively. Interference was within 6.5% when bilirubin (300 mg/L), hemoglobin (5 g/L) or chyle (1,930 formazine turbidity units) was added to serum samples with known IgGSc concentrations. Linearity was assessed by assay of serially diluted serum samples and comparison of expected with measured results by regression analysis: IgG1: $y=1.006x - 9.4$, $r=1.000$. IgG2: $y=0.998x - 5.2$, $r=0.999$. IgG3: $y=0.994x + 0.14$, $r=0.999$. IgG4: $y=1.012x + 0.13$, $r=0.999$. All assays

were linear over the range tested. Comparison was made with the TBS IgGSc assays for the Dade Behring BN II. Serum samples from normal subjects ($n=100$) were assayed for IgGSc on both systems and regression analysis of the results shows good agreement: IgG1 $y=1.02x+2.2$ ($r=1.00$); IgG2 $y=1.04x+2.3$ ($r=1.00$); IgG3 $y=0.94x-0.15$ ($r=0.94$); IgG4 $y=1.22x-0.36$ ($r=1.00$). The IgGSc assays for the Roche Modular P provide a rapid, precise method of measuring IgGSc in serum and show good agreement with existing assays.

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Should anti tissue transglutaminase alone be used for the serological detection of coeliac disease?

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Oral Presentation

Increasingly anti tissue transglutaminase antibodies are being used to detect coeliac disease. Here we address the question as to whether they should be used alone or in combination with other serological tests.

1364 consecutive samples received in one year from the gastroenterology department for coeliac serology were analysed for IgA and IgG antibodies to gliadin, IgA antibodies to endomysium and IgA antibodies to tissue transglutaminase (TTG). The pathology computer was searched for histology results of small intestinal biopsy.

1180 samples were negative for all antibodies.

Of the 21 samples with negative endomysial and with TTG of above 3, histology was performed on 16. Ten of these showed active CD, 5 showed treated CD and only one was normal. The specificities of the 2 tests are virtually identical as all samples with a TTG below 4 were negative for anti endomysial antibodies.

Of the 36 samples with positive IgA gliadin and negative TTG, biopsies were performed on 21. Seventeen of these were normal, 3 were known coeliacs on treatment and 1 was suggestive of coeliac disease. Of the 30 samples with positive TTG and negative IgA gliadin, biopsies were performed on 20. Of these 18 had active coeliac disease, 1 had treated coeliac disease and one was normal.

Of the 21 samples with raised IgG gliadin and normal TTG, biopsies were performed on only 7. Four of these were normal and three were known to have coeliac disease and were on treatment.

Conclusions: 1) Anti TTG is more sensitive and more specific than either IgA anti gliadin or anti endomysial. 2) A raised IgG anti gliadin alone did not detect any additional cases of coeliac disease. 3) Measurement of anti TTG alone has the best sensitivity and specificity for the detection of active coeliac disease of any combination of serological tests.

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Assessment of quantitative proteinuria using random urine protein: creatinine index at different times of the day

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The objective of this study was to determine the usefulness of measuring protein:creatinine index (PCI) in random urine specimen as an alternative to 24 hour urine protein excretion.

The study is a prospective study for 7 months commencing from August 1994. Measurement of PCI in 3 random urine specimens collected at 08.00, 12.00 and 16.00 hours were compared with daily urine protein excretion. The participants included a total of 250 individuals including 125 healthy control subjects (100 aged >15 years, 25 aged <15 years) and 125 patients with proteinuria who were subdivided according to age (100 aged >15 years, 25 aged <15 years), renal function (69 with normal renal function, 56 with impaired renal function) or protein excretion (61 with proteinuria <1.0 g/day, 64 with proteinuria >1.0 g/day). The main outcome measures comprised 24 hour urine protein excretion (P24) including that corrected according to surface area (P24/1.73), protein/creatinine index in 24 hour specimen (24PC) and in random specimens of morning (PC8), midday (PC12) and afternoon (PC16). Student's unpaired t-test, analysis of variance (F test) and linear regression analysis were used for data evaluation.

Results: Numerical similarity was noted in mean values of 24 PC, PC8, 12, 16 with P24 and P24/1.73. Correlation studies involving all subjects (n=250) revealed highly significant correlation ($p < 0.001$) between random PCI and each of 24 PC ($r = 0.978$), P24 ($r = 0.889$) and P24/1.73 ($r = 0.912$). The correlations were higher in proteinuric patients, irrespective of age, renal function and degree of proteinuria, than in healthy subjects, and with indices of random specimens obtained at 12.00 and 16.00 than that at 08.00 hours. The PCI of random urine specimen is strongly related to timed protein excretion making its measurement a suitable and convenient alternative to timed collection particularly for follow-up and screening purposes.

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The interference of IgM monoclonal components in the Szasz method for the determination of GGT on the Olympus AU2700

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Haemolysis interference in the Szasz GGT method has been well documented. Here we present a novel finding

of an additional interfering factor - IgM monoclonal components.

Following routine serum analysis in a 56-year-old woman, a GGT (Szasz - Olympus AU2700) result of -39 U/L was obtained. Repeat analysis confirmed this initial result. On dilution with distilled water, a turbid precipitant formed. Another dilution was carried out with saline from which no precipitant formed and GGT analysis (Szasz - Olympus AU2700) produced a result of 30 U/L.

As the Szasz method is no longer commercially available, GGT analysis was also carried out on this specimen using the CE marked IFCC method. Similar results were obtained on the undiluted serum (-39 -44 U/L compared with -39 U/L).

Repeat analysis on a Vitros 950 gave a result (neat) of 28 U/L, suggesting that the separating layer of the dry-slide technology removed the interfering substance prior to analysis.

Capillary zone electrophoresis and immunofixation revealed a single IgM-lambda monoclonal band of 4 g/L.

A small preliminary study of patients with IgM monoclonal components has revealed a similar pattern when the serum is analysed neat and diluted for GGT. However, these results have yet to be confirmed and the cohort needs to be increased.

This case tentatively suggests that IgM monoclonal components may be an additional interfering factor of the Szasz and IFCC GGT methods on the Olympus AU2700.

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Rationalising the protein laboratory in a busy teaching hospital

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The majority of analyses performed in the protein laboratory investigate paraproteinaemia and diagnosis and monitoring of B cell malignancies. This demands thorough investigation and prompt reporting.

In 2003 the protein laboratory at City Hospital Birmingham performed 1500 protein electrophoresis (PE), 12,000 immunoglobulin analysis, 330 serum immunofixation tests, and 100 densitometric scans of monoclonal bands. There were over 200 tests for Bence Jones Protein and 150 urine immunofixation tests. The turn-round averaged about 2 weeks.

To simplify workflow and improve efficiency, every aspect of the process was examined and result reporting reviewed. A new single worksheet was created for both PE and immunoglobulins which includes results for albumin, total protein and densitometric scanning of monoclonal bands. For each investigation the last 5 results are shown making it easier to identify patients

requiring further investigations, which are automatically added by the computer.

The number of interpretative comments for PE was reduced from 22 to 6. An audit of 2 months work revealed that of 55 serum immunofixation tests performed on minimal monoclonal bands identified by PE, only 13 were investigated further. As a result the comment, 'minimal protein band consider repeat in 6 months', was introduced. Serum immunofixation is then performed on the repeat sample if the minimal band persists.

Previously, there were 45 interpretive comments for immunoglobulin results, which was confusing for staff and clinicians. Cumulative reporting of the last 5 patient results overcame the need for these. Immunoglobulin analysis is now performed in real time.

After the review, turn-round of patient investigations using the new computerised single worksheet has improved significantly from 12 to 2 days. A similar approach to Bence Jones protein analysis has also improved turn-round from 3 weeks to <1 week. Despite initial reservations, changes have been received favourably by laboratory staff and clinicians alike.

81 Two unusual cases of transient hyperphosphatasaemia

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Two non-pregnant patients with no evidence of liver or bone disease presented with very high alkaline phosphatases (ALP), and were investigated to determine the origin of the enzyme.

Alkaline phosphatases are determined as part of a liver/bone profile, on the Olympus AU640. Isoenzymes are investigated by electrophoresis on cellulose acetate, with pH 10 barbitone buffer. Visualisation uses bromochloro-indolyl phosphate/nitro blue tetrazolium salt, in 2% agarose.

Patient EC is a 67 years old male myeloma patient on a plateau, not on treatment, with his M band at about 30 g/L, and with severe immune paresis. He is reasonably healthy, and reported no problems prior to showing an unexpected ALP rise from 45 to 1650. The isoenzymes showed a typical transient hyperphosphatasaemia pattern, which disappeared over the next 2 months.

Patient MH is a premature baby aged 10 days, with periodic episodes of sepsis, and having daily LFTs. His ALP rose from 180 to 1400 over 5 days, without any deterioration in his general condition. The electrophoresis showed no bands whatsoever, but a general staining across the whole electrophoretogram. The ALP returned to normal over 10 days, with a disappearance of the

general staining, and a reappearance of the expected bone isoenzyme picture.

This department assayed about 90 specimens this year for isoenzymes of ALP, of which 10 had transient hyperphosphatasaemia. These bands were usually found in well children attending outpatients.

EC is unusual in being an adult, where very few transients are seen.

MH is unusual in that the very characteristic transient pattern was not seen.

It is important to recognise that hyperphosphatasaemia does not necessarily indicate bone or liver disease. Electrophoresis of isoenzymes is an important tool to eliminate other investigations.

82 A study of the immunoreactivity of caeruloplasmin using a resonant mirror technology

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Oral Presentation

The aim of this study was to investigate the immunoreactivity of caeruloplasmin from reference material compared with caeruloplasmin from fresh patient serum.

Caeruloplasmin assays can show considerable method dependant variation. Recent work highlights an example of this phenomenon. Fresh UKNEQAS samples gave readings of 0.479 g/L by nephelometry and 0.368 g/L by turbidimetry. When retested 4 months later the same samples gave readings of 0.449 g/L by nephelometry and 0.413 g/L by turbidimetry. This implies that the caeruloplasmin changes its immunoreactivity over time, perhaps related to a change in structure.

To test this theory, resonant mirror technology was used to measure the kinetic parameters of the antibody binding reaction to caeruloplasmin. This was performed on an IASys (ThermoLabsystems). Four biosensors were produced by immobilizing antibodies from four sources on the reactive surface of carboxymethyl dextran cuvettes. When caeruloplasmin is added to the biosensor, the binding reaction to the antibody is monitored through the interaction of the Ab-Ag complex with an evanescent wave on the reactive surface of the cuvette. The kinetic parameters measured were k_a , k_d and affinity. Two different reference serum samples representing aged caeruloplasmin and two different patient serum samples representing fresh caeruloplasmin were analysed by the four different biosensors.

The four different biosensors showed different kinetic characteristics due to the different antibodies used. In all cases, the patient serum showed at least a 10-fold

reduction in affinity compared with aged caeruloplasmin for a given biosensor. For all biosensors, the average affinity for reference serum was 2.66×10^{-14} and 1.23×10^{-12} for patient sera ($p < 0.05$).

There was a clear difference in the immunoreactivity of caeruloplasmin from aged and fresh samples to a given antibody. This indicates a change in the epitope expression and hence a change in the protein structure. This would explain why the results for UK NEQAS samples appear different on retest after 4 months.

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Measurement of caeruloplasmin on the Olympus AU400®, AU640®, and AU2700® chemistry immunoanalysers

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Plasma caeruloplasmin concentrations are reduced (dialysable copper concentration increases concomitantly) where Wilson's disease is present. Low levels are also found in malnutrition, malabsorption, nephrosis and biliary cirrhosis. Increased caeruloplasmin levels are noted in diseases of the reticuloendothelial system. The Olympus assay (OSR6164) is a liquid-stable automated homogeneous immunoassay which measures serum or heparinized plasma caeruloplasmin levels quantitatively. We have evaluated the application of this reagent on the AU400, AU640, and AU2700 chemistry immunoanalysers.

The assay uses 2 µL sample mixed with 180 µL R1 accelerator buffer and incubated for approximately 5 minutes. R2 reagent (50 µL) containing anti-human caeruloplasmin antiserum is then added. Turbidimetric measurement of any resultant complexes formed during the antigen-antibody reaction proceeds by measuring absorbance bichromatically at 340/800 nm at 37°C for approximately 5 minutes. The reaction is a calibrated endpoint method, utilizing 5 liquid stable multicalibrators (ODR3023) traceable to CRM 470/RPPHS. The assay displays a linear measurable range of 60-2000 mg/L and prozone hook effect better than 9000 mg/L. Imprecision CV values are <2% for within-run and <5% total CV for all analyzers tested (120-1810 mg/L) using NCCLS EP5-T2. Lipaemic, haemolytic, icteric and rheumatoid interferences are all <5% at elevated levels (1000 mg/dL intralipid, 5 g/l haemoglobin, 684 µL bilirubin, 11.4 mmol/l triacylglycerol, 750 IU/mL RF) of each (NCCLS EP7-P). Reagent displays 60 day on board stability with recalibration necessary only every 14 days. Excellent correlation of the assay was obtained on AU640 compared to Dade-Behring nephelometry (BNII)®, AU400 and AU2700. [Y [AU640]=

1.020[BNII]®]-3.5; $r=0.989$, $n=50$; range: 90.0-510.0mg/L]. [Y [AU640]=0.964 [AU400]+ 13.2; $r=0.999$, $n=80$; range: 87.0-2071.0 mg/L]. [Y [AU640]=1.0002[AU2700]+1.0; $r=0.999$, $n=80$; range: 87.0-2071.0 mg/L]

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Measurement of cerebrospinal fluid parameters on the Olympus AU640®, AU400® and AU2700/5400® analysers

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Analysis of cerebrospinal fluid (CSF) is important in the diagnosis and management of patients with various infectious diseases, neoplasia, stroke, trauma and dementia. The purpose of this study was to develop analyser applications on the Olympus platform suitable for the quantitative determination of glucose, albumin and IgG in cerebrospinal fluid. Reagents evaluated were from the Olympus menu which prior to this had been used for serum or urine determinations. The methodologies were as follows: glucose [hexokinase]; albumin [immunoturbidimetric assay for albumin]; IgG [immunoturbidimetric assay for IgG]

Imprecision studies on patient samples and controls resulted in the following. For glucose within run and total imprecision was <3.0%. For albumin and IgG within run imprecision was <5% and total imprecision was <10%.

Linearity checks were performed according to NCCLS EP6-P protocol. Reportable ranges for the three analytes are as follows: glucose: 10.0-800 mg/dL; albumin 0.5-30.0 mg/dL (0.05-0.30 g/L); IgG 2.0-45.0 mg/dL (0.02-0.45 g/L).

Haemolytic and icteric interference was evaluated according to NCCLS EP7-P. In all cases there was no significant effect (<10%) up to 500 mg/dL of haemolysate. For glucose and microalbumin there was no significant effect (<10%) up to 40 mg/dL bilirubin. In the case of IgG a positive interference of >10% was noted at 12 mg/dL bilirubin.

Method comparison versus a routinely used competitor method (Roche®) was carried out using 100 patient samples. The results show good correlation as follows. IgG $Y=1.00x - 0.41$, (r) = 0.97, glucose $Y= 0.97x + 0.31$ (r) = 0.991, albumin $Y= 1.07x - 1.24$ (r) = 0.99.

In conclusion these three applications are suitable for CSF applications on the Olympus series of analysers. Xanthochromic or icteric samples should be avoided for IgG determinations.

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The first CE marked NHS in-house assay: the central role of the technical file

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In 2003 the Medical and Healthcare Products Regulatory Agency (MHRA) issued guidance on CE marking NHS in-house assays to comply with the in-vitro diagnostics legislation. Although further advice is awaited, clearly some NHS in-house assays will require CE marking.

We recently developed an in-house assay for thiopurine S-methyltransferase. This complex assay involves a number of steps and the manufacture of in-house reagents, making it ideal to check out the work involved in complying with CE marking.

We classified our assay as low risk, therefore requiring self-certification culminating in a Declaration of Conformity.

Our technical file contains the required documentation, divided into five sections. Essential requirements are a list of minimum safety requirements and how the vigilance and reporting schemes operate. Other sections provide evidence of how this has been achieved; Failure Mode and Effects Analysis (FMEA), Method Operation, Method Development and User Information and Feedback.

For the FMEA we created an Excel spreadsheet including a detailed assessment of potential failure mechanisms, incidence, severity, and measures taken to minimise these. Thirty-four risk areas were identified, thirteen requiring further action and also provided the evidence for the purchase of a new analyser with a bi-directional interface to the laboratory computer!

The technical file is constantly updated. In the two months since the assay was CE marked: the SOP has been updated twice; three batches of reagents were discarded, as they failed our new quality standards; the water-bath was replaced with a hot-block; there have been two updates of the user information leaflet; fourteen separate contacts with users logged, from reporting deficient results, to stability in samples delayed over Christmas/New Year.

Regardless of any further MHRA guidance we have learnt a lot from CE marking our assay. By applying what we have learnt to other in-house tests we can only improve our laboratory service.

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Consultant and biomedical scientist workload in clinical biochemistry

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Concerns have been raised over the growing pressures on staff in clinical biochemistry, and the impact this may

have on them and their profession (NHS Clinical Biochemistry - A Profession Under Siege (2002)). This study investigated how workload has changed for consultants (medical and clinical scientists) and biomedical scientists (BMS) in clinical biochemistry over a more recent two-year period (2000/2001-2002/2003).

Data was obtained from Trusts participating in the National Pathology Alliance Biochemistry Benchmarking Review in both 2000/2001 and 2002/2003 (n=21). The review is voluntary and Trusts pay to participate. Trusts provided data on staff numbers and type in clinical biochemistry, and the number of requests and tests performed by the department. The data was analysed in SPSS and all values are given as the mean.

The number of consultants and BMS staff per department did not change significantly between 2000/2001 and 2002/2003 (consultants, 2.5 vs 2.5; BMS, 23.1 vs 23.8). In contrast, the number of requests per department increased from 400,251 to 449,278 (p<0.01), a rise of 12%. Similarly the number of tests per department increased by 18%, from 2,682,895 to 3,171,153 (p<0.01). The number of requests and tests per consultant rose from 192,351 to 218,639 for requests and 1,341,444 to 1,593,637 for tests (p<0.01). A similar trend was seen for BMS (requests per BMS, 18,277 vs 19,844; tests per BMS, 123,947 vs 140,802; p<0.01).

The benchmarking reviews have indicated a rising workload over a number of years. This is illustrated in these laboratories participating in 2000/2001 and 2002/2003. However, the numbers of consultants and BMS staff have remained unchanged. Such increases in workload highlights the increasing pressures staff are under in clinical biochemistry, potentially compromising workforce satisfaction and health, as well as patient care.

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Critical incident reporting in NHS pathology laboratories

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The National Pathology Alliance Benchmarking Review (the review) has collected critical incident numbers for five years from participating laboratories. Numbers of incidents have risen over this period.

This data has been criticised because the definition of a critical incident varies between laboratories.

The aim was to: 1) collect and analyse definitions of critical incidents and look for variations and 2) use the definitions to identify common themes that might be used in a standard definition.

The review operates annually gathering data from UK

pathology laboratories. Data for this study was collected by questionnaire covering pathology for 2002/2003.

Questions: 1) Is the number of critical incidents involving pathology separately recorded? 2) Explain how a critical incident is defined, a text response 3) How many critical incidents per year?

Numerical analysis was performed on questions 1 and 3. Laboratories providing data for two years were used to identify trends. Question two was analysed using text content analysis. Themes were identified and used to generate the constituent elements of a critical incident.

Possible cause of bias: participation in the review is voluntary and there is a charge for taking part.

Results: 51/60 laboratories provided data. 78 per cent of laboratories recorded critical incidents involving pathology. Key elements were:

Harm to persons (staff, patient or other) or adversely affecting patient care (35), an incident resulting in damage to Trust property (13), an event resulting in adverse media attention (6). Five further elements occurred in at least 3 definitions each and six occurred in one definition only.

Critical incident numbers over two years showed little change.

Conclusion: Under clinical governance critical incidents should be recorded although there is little standardisation. If CPA is mandatory and Trusts are to be compared and patients are to have choice in where they receive treatment, such issues will become more pressing.

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Predicting the future performance of a clinical biochemistry laboratory by computer simulation

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In recent years, the number of samples which clinical biochemistry laboratories must handle has been increasing rapidly. At our hospital, a medium sized general hospital covering a population of 235,000, a 20% cumulative increase in workload is being observed. How this will affect the response time of the laboratory is the subject of this study.

A model for the analytical process was constructed which was then validated using data gathered from the laboratory. The computer simulation (using the software package SIMUL8 (Hauge J W, Paige K N, Learning SIMUL*: The Complete Guide, ClearVu Publishers, Bellingham, 2001)) was used to predict the effects of increased workload, replacement of analytical instruments and changes in working practices.

The primary conclusion of this study is that resources must be invested in the laboratory if it is to maintain a satisfactory level of service. The model demonstrates that the investment must be balanced if its full potential is to be realised - faster analysers and automated sample handling must be accompanied by appropriate staffing, computing resources, efficient data input and changes in work practices. It has been demonstrated from the prediction that the laboratory can deteriorate from providing a satisfactory service to one that could not be tolerated, at an alarming rate. Continued use of the simulation will require periodic updates, to reflect new operating conditions, and progressive model refinement to improve its accuracy.

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A virtual laboratory tour: an alternative way of promoting laboratory medicine

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The two key aspects of an efficient, modern NHS laboratory are the staff and the actual laboratories. We are all aware of high-flying staff with a national/international reputation who are willing to share their expertise, especially through publications and by giving presentations at scientific meetings. This often helps laboratories to develop state-of-the-art services either de novo, or by upgrading existing services. We can also learn a lot by visiting laboratories when redesigning our own laboratory, when introducing new equipment, or we can use a laboratory tour as an educational tool for service users. However, this can be time consuming and cause disruption to the host laboratory.

As a way of promoting laboratory medicine at Hull Royal Infirmary, we have produced a virtual laboratory tour, which is available on the Trust Website (www.hey.nhs.uk/biochemistry). The aim is to allow visitors to the Website, who may be healthcare professionals or members of the public, to view the laboratories and see the facilities that are available in Hull. The current format of our virtual tour is very simple, but the production of a more detailed version could be tailored according to need, for example to give an educational tour for service users, or for the promotion of healthcare scientists. This would overcome the physical barrier of actually visiting the laboratory. In this context, we are using this virtual tour in conjunction with job advertisements. The aim of this is to share our enthusiasm for working in Hull by encouraging potential applicants to view what we consider to be modern, state-of-the-art laboratories, without any travelling or commitment.

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An evaluation of Abbott Diagnostics inventory management system

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The aim of the study was to evaluate an inventory management system supplied by Abbott Diagnostics (ADIMS), and to aid with tailoring the package for use in the UK.

In September 2002 the department was invited by Abbott Diagnostics to attend a user forum into inventory management. Prior to the meeting our current processes were audited, and the following issues were raised: ordering delays, resulting in large stock being held; limited stock management records; CPA compliance.

This resulted in reagents being used very close to expiry dates, there were also CPA compliance issues particularly with stock inventory records.

At the forum we agreed with Abbott Diagnostics that we would pilot a CoMed software product currently available in mainland Europe, with a view to recommending modifications for use in the UK.

The system is a complete ordering and reagent management package, which monitors reagent usage through individual pack ID barcodes and a handheld scanner, and includes direct ordering via EDI.

The system was installed in January 2003 in the Acute Biochemistry Section for use with reagents used on the twin Abbott Aerosets, direct ordering via EDI to Abbott Diagnostics was also set up at this point.

The system was used live for complete reagent management including: electronic ordering, reagent delivery tracking, reagent usage logging and stock control.

During the evaluation a number of recommendations were made including instrument specific reagent logging and low stock alarm warnings.

In conclusion the system was found to be user friendly with good staff compliance. The main advantage is that the system allows for CPA compliance under sections D.3 subsections D.3.1, D.3.2.a, c, d and D.3.3. We have now significantly reduced stocks held within the section as orders are now received within 5 working days instead of up to 4 weeks.

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Causes of false positive reports for drugs of abuse in urine

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Analyses used to detect drugs of abuse in urine are configured to minimise the number of false positive results

at the expense of increases in false negative results because of the potential imposition of sanctions following a false positive report be it in a clinical, workplace or prison setting. The number of false positive reports made by participants in the United Kingdom National External Quality Assessment Scheme (UKNEQAS) for drugs of abuse in urine has remained relatively constant for some years despite improvements and technological advances in both immunoassay and chromatographic procedures used for drug analysis. We have investigated the cause of these false positive reports for the 12 samples distributed during 2003. From the 196 to 203 laboratories reporting on each sample, a total of 93 laboratories were requested to report by means of a questionnaire the cause of a total of 164 false positive reports. In this ongoing survey, 84 responses (51%) have been received.

The laboratories categorised the false positive reports to be the result of analytical errors (31%), non-analytical errors (30%), errors of interpretation (26%), or a combination of analytical and interpretation errors (4%). Ten percent of cases were not categorised. The commonest cause of non-analytical error (number of reports) were transcription errors completing forms (18) with several laboratories noting that patient data are transmitted electronically. Seven laboratories reported the wrong data and 1 switched samples. Errors traced to immunoassay data resulted from cross-reactivity with related compounds (4), unexplained false positives (3), cocaine metabolite reported as cocaine (3), and difficult to read near-patient devices (5). For chromatographic procedures there was chemical change either on-column or during preliminary acid hydrolysis (8), false identification of closely running TLC spots (8), of compounds with similar retention times (8), and of compounds with similar mass spectra (2). There was carry-over between samples (4), reporting of trace (below cut-off) concentrations (3), and one case of contamination during sample work-up. In several cases of analytical error, laboratories reported that procedures had been adapted to prevent a recurrence of the error.

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Trueness of progesterone assays: results of the latest UK NEQAS ID-GCMS exercise

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ID-GCMS targeting exercises have been a feature of the Steroid Schemes from their inception. They show the relationship between the ALTM / method group medians and a reference method value, so that "true" bias may be established. This provides participants and industry with a benchmark against which the analytical validity of methods may be assessed.

Three pools were targetted at the Steroid Reference Laboratory of Professor Linda Thienpont (Gent, Belgium) for ID-GCMS analysis according to established protocols for reference value certification. Triplicate analyses on three separate occasions were undertaken.

Data were extracted for each of the above samples and obvious outliers removed. 5th, 25th, 50th, 75th and 95th centiles were calculated for the whole data set and for each method. Absolute and percentage differences for each data point were calculated and graphed. The slope of the regression line and its linearity, the intercept of the regression line and the spread of data between users provide information on calibration, specificity and robustness.

The validity of the ALTM as an appropriate target value was confirmed, in that its relationship with the GCMS target is close to unity and its intercept is small. The regression slopes of most methods were within 10% either side of unity; only Wallac Delfia had a major calibration error. Intercepts varied amongst methods; Abbott AxSYM, Bayer Advia Centaur, Bayer Immuno1 and Wallac Delfia had intercepts >2.0 nmol/L. Methods exhibited different group characteristics, with tight grouping for Abbott AxSYM, Roche Elecsys, Roche E170 Modular and Wallac Delfia, and higher spread for Bayer Advia Centaur, Immulite 2000 and Tosoh AIA.

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Do interpretative comments work?

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In order to assess the efficacy of our interpretative comments in general practice, we reviewed a particular set of 3 coded comments which are appended to those thyroid results suggestive of sub-clinical hypothyroidism. When the TSH exceeds the upper limit of normal, but the results are not overtly hypothyroid, with a normal or low/normal fT4 and when the clinical details do not indicate any thyroid medication, the comments suggest repeating thyroid function tests with thyroid peroxidase antibody in 2 months.

Over a six month period, all TFTs from GPs with these comments were collated and a retrospective investigation of follow-up tests was undertaken. A control group of similar samples was identified with no comments appended.

A positive response was observed in 71% of cases with a variety of actions ranging from: repeat TFT with TPO in 2 months time (as suggested: 73% of responders) repeat TFT at various time intervals (14%); straight onto thyroxine replacement (13%)

Some surgeries were apparently more responsive to the appended comments than others. Responses from different GPs also varied.

In the control group with no comments appended, follow-up testing occurred in only 38% of cases (with repeat TFTs requested), 28% of patients were commenced straight onto thyroxine and only 2% requested a TPO.

From this evidence we can see that comments do influence patient follow-up and management.

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National guidelines for analysis of CSF bilirubin and their impact

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Subarachnoid haemorrhage (SAH) is spontaneous arterial bleeding into the subarachnoid space, usually from a cerebral aneurysm. The detection of SAH in patients is vital to determine whether an angiography and preventative surgery is required. An increase in cerebrospinal fluid (CSF) bilirubin is the key finding which supports the occurrence of SAH but it is not specific. The Specialist Advisory Group for EQA of CSF Proteins and Biochemistry published National guidelines for analysis of CSF bilirubin in suspected SAH patients. A stable artificial matrix has been formulated for the UK NEQAS scheme and recovery data indicates that spectrophotometry is accurate and linear for bilirubin concentrations within the working range. The results obtained suggest that these guidelines have had a major impact on current practice in UK diagnostic laboratories for detection of CSF bilirubin. The guidelines state that spectrophotometry should be used in preference to visual inspection to detect CSF bilirubin and decrease false negative results. Prior to the production of the guidelines 24/101 (24%) laboratories used visual inspection and 77/101 (76%) used spectrophotometry. The approximated error rate within the EQA scheme at this time in bilirubin detection was 40%. Since the publication of the guidelines 108/115 (94%) laboratories now use spectrophotometry and only 7/115 (6%) still use visual inspection. The sharp decline in laboratories using visual inspection for detection of CSF bilirubin coincides with a decreased error rate, currently 9%. We conclude that the publication of National guidelines has significantly improved the detection of CSF bilirubin, which should improve the clinical detection of SAH.

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The haemolysis challenge

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Haemolysis can occur for a number of reasons, one of which is venepuncture technique. Although steps can be taken to minimise haemolysis, it is sometimes unavoidable and may produce erroneous results in some biochemical assays. The prudent laboratory must be able to identify haemolysis and be aware of the potential analytical interferences.

Serum pools were spiked with haemolysate to produce Hb concentrations ranging from 0-10 g/L. Classification of the haemolysed samples by laboratory staff was tested by way of a challenge: staff were asked to classify 'spiked' samples as normal, slightly, moderately or grossly haemolysed. The effect of haemolysis was tested on a number of common analytes on an Olympus AU640 analyser in blood collected into SST tubes. Changes in values were considered significant ($p=0.05$) if greater than $2.8 \times SD$ (analytical + intra-individual). To determine the effect of venepuncture technique, an audit was carried out to compare the incidence of haemolysis in Phlebotomy (controlled setting) and A&E (emergency setting).

All unhaemolysed samples were correctly recognised as were those above 4 g/L Hb (grossly haemolysed). Moderate and slightly haemolysed samples were poorly identified, and solutions containing 2 g/L Hb were rarely (12.5% of the time) considered to be grossly haemolysed. Generally there was an underestimation of the degree of haemolysis.

At Hb concentrations of 0.125 g/L or less, none of the analytes tested were affected. At 0.25-0.5 g/L (slight haemolysis) only AST was affected. Moderate haemolysis of 1 g/L also affected CK and CKMB. Gross haemolysis of 2 g/L or more affected all analytes tested.

0.36% of samples taken in the phlebotomy department (2 of 549) were haemolysed compared to 3.09% of samples taken in A&E (17 of 550). The delay in providing accurate results when the first sample was haemolysed could delay patient care and add to the 'trolley wait' times.

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Delayed separation revisited

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The devolvement of clinical services to Primary Care and the concept of taking pathology nearer the patient with phlebotomy services in GP surgeries means that delays from sampling to analysis are inevitable. In view of

increased use of Clinical Biochemistry testing in Primary Care we have reassessed the stability of analytes (at room temperature and 4°C) on the Olympus AU640 and Abbott Architect i4000. Blood collected into SST tubes was stored unseparated for up to 7 days before centrifugation and analysis. Changes in values were considered significant ($p=0.05$) if greater than $2.8 \times SD$ (analytical + intra-individual variation).

When samples were left unseparated for 24 h at room temperature, the following analytes were significantly affected: calcium, phosphate, glucose, lactate, magnesium, ammonia and free T3. Potassium, chloride, cholesterol, HDL and immunoglobulins were additionally affected after three days delay in separation, and sodium after seven days. Ethanol, HCG, PSA, CEA and AFP were not elevated in the test samples and are therefore regarded as untested.

When left unseparated for 24 hours at 4°C, the following analytes were significantly affected: potassium, calcium, magnesium, phosphate, HDL and immunoglobulins.

This study enabled us to devise a protocol outlining acceptable tests for unspun samples received after an overnight delay, for example from a late-running clinic at an out-reach centre. Lipid profiles, thyroid function tests, gonadotrophins and ferritin are all acceptable. Although statistically affected due to low intraindividual variation, changes in free T3, HDL and immunoglobulins are probably not clinically significant, therefore these tests can be accepted. Samples unspun overnight are unsuitable for potassium, calcium, phosphate, magnesium, glucose, lactate and ammonia.

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Is assay bias in the UK NEQAS for serum cortisol influenced by the sex of the donor?

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The United Kingdom Quality Assurance Scheme (UK NEQAS) for serum cortisol shows the Roche Elecsys assay having a positive bias, related to cortisol concentration in female, but not male, pooled samples. Over the period 29.10.02-16.9.03, for cortisol samples with an all method mean 400-500 nmol/L, the bias for the Elecsys assay $\{(Elecsys \text{ method mean} / \text{all method mean}) \times 100\}$ for female samples was 3.2-17.5% ($n=12$) and for male samples -6.5 to 1.5% ($n=17$). The correlation between bias (y) and all method mean cortisol (x) was $y=0.23x - 38$ in female samples and $y=0.03x - 15$ in male samples. The Bayer Immuno-1 assay behaves similarly to the Elecsys, although the difference is less marked; the Perkin-Elmer Wallac DELFIA assay shows no difference in bias for female or male

serum pools, but has an assay bias inversely related to cortisol concentration.

To determine whether the results observed in the UK NEQAS could be reproduced we measured serum cortisol in male and female subjects by Elecsys and DELFIA assay, using untreated serum collected into Beckton Dickinson serum separation tubes. With samples having cortisol levels between 200-600 nmol/L the Elecsys assay gave higher results than DELFIA. In male samples the mean difference (n=11) was 55 nmol/L (range -27 to 179) and in non-pregnant female samples (n= 17) 46 nmol/L (range -32 to 87); there was no difference in female subjects judged to be pre- or post- menopausal on the basis of age. In pregnant females the mean difference between the Elecsys and DELFIA results was greater, 91 nmol/L, (range -19 to 138).

These results suggest that samples distributed in the UK NEQAS cortisol scheme may not behave like patient samples.

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Impact of obtaining signed consent for newborn screening tests in Scotland

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In February 2003, the newborn screening programme in Scotland introduced a form for parents to sign giving consent for newborn bloodspot tests. An information leaflet is given to parents prior to a home visit from the community midwife who explains the nature of the tests, answers any questions and obtains consent to collect the heel-stab capillary blood specimen. The form offers the parents a choice of screening tests and asks for consent to store the bloodspot card and to use any residual spots for anonymous studies e.g. HIV testing. The format is a series of tick boxes: I wish, and I do not wish my baby to be tested for phenylketonuria (PKU); and I wish, and I do not wish my baby to be tested for congenital hypothyroidism (CHT; I wish, I do not wish my baby to be tested for cystic fibrosis (CF) and I agree, and I do not agree to the storage of the blood spot card beyond the 12 month testing period; I agree, and I do not agree to the use of any left over blood spots for anonymised research.

Preliminary analysis indicates that there has been a rise in the number of parents withholding consent for all newborn screening testing. The number of refusals for all tests in 2000, 2001 and 2002 were 15 (0.027%), 20 (0.038%) and 18 (0.034%) respectively while for the first 9 months of 2003 (45,523 specimens), 33 parents (0.072%) declined all of the tests. There were a further 8 parents who declined only CF testing and 1 parent who refused CF and CHT testing increasing the refusal

rate for any test to 0.092%. Figures for refusal for storage were 45 (0.1%) and for anonymous studies 854 (2%). Thus the introduction of written consent for newborn screening has resulted in a small but unwelcome decrease in the uptake of testing. Methods by which the refusal rate can be minimised are under consideration.

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The introduction of cystic fibrosis testing into an established newborn screening programme in Scotland

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Bloodspot testing for cystic fibrosis (CF) by Delfia immunoreactive trypsinogen (IRT) assay was introduced into the Scottish newborn screening programme in February 2003. An IRT-DNA-IRT protocol is used. All specimens with an IRT level ≥ 99.5 th centile are investigated for the 31 most common CF mutations in the Scottish population using the PE Applied Biosystems oligonucleotide ligation (OLA) assay. The 31 mutations tested which, include 90% of the CF alleles in the Scottish population, are G85E, R117H, Y122X, 621+1G>T, 711+1 G>T, 1078 del T, R334W, R347P, R347H, A455E, Q493X, Δ I507, Δ F508, V520E, 1717-1 G>A, G542X, S549R, S549N, G551D, R553X, R560T, 1898+1G>A, 2183 AA>G, 2789+5 G>A, R1162X, 3659 del C, 3849+10kb C>T, 3849+4 A>G, W1282X, 3905 ins T, and N1303K. Babies with 2 mutations are referred to a specialist CF physician. Babies with 1 mutation and babies from minority ethnic groups with an initial raised IRT are followed up through IRT testing of a second specimen obtained at 27 days. Babies with a second raised IRT are sweat tested. In the first 9 months of the programme, 41,019 babies have been tested and 255 identified with a raised IRT (0.62%). Subsequent mutation analysis has identified 14 cases of CF with either 2 mutations (13) or 1 mutation and a positive sweat test (1) (CF incidence 1:2900). Probable carrier status has been identified through screening in a further 19 infants and the families offered genetic counselling.

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An unusual case of excessive potassium variation resolved by gentamicin treatment

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Faith, Hope and Charity are three independent hardworking 5 year-old Beckman LX20s, employed in a routine (24/7) biochemistry automated section, used to handling a combined workload of around 14,000 tests per day.

In January 2003, Charity's potassium precision was seen to deteriorate markedly (CV 0.8%-2.3% at a level of 4.5 mmol/L) After a period of rest (standby) she was unable to measure potassium due to excessive reference drift, but after several measurement cycles would become asymptomatic when running continuously.

Conventional investigations failed to identify her problem, which was eventually considered to be caused by 'contamination'. Treatment consisted of a time consuming decontamination procedure to clear the system of possible contaminating agents. The procedure was effective but after only a few weeks, her symptoms recurred.

Further studies involving recording raw data from the potassium ion selective electrode, and comparisons with identical instruments, indicated a problem common to the reference measurement cycle. This prompted further examination and a series of swabs were taken (aseptically) from various reagents and sites within Charity in order to locate and identify a possible bacterial infection.

Results revealed her to have an isolated and abundant growth of *Pseudomonas aeruginosa* in the port of the reference electrode, a finding consistent with the raw electrode data. The organism was known (in pathological cases) to be sensitive to gentamicin.

Flushing Charity's system with gentamicin added to the electrolyte reference solution (adjusted to a therapeutic level) had an almost immediate and lasting effect in restoring reliable potassium measurement.

The source of contamination and exact mechanism of the disturbance is unknown, but daily potassium CVs are carefully monitored for relapse and appropriate remedial action with gentamicin is taken when necessary.

To our knowledge this condition is previously unreported.

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Biomedical Science Education in Singapore

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Our aim was to describe a novel full-time clinical hospital attachment for diploma students.

The NUH-Ngee Ann Polytechnic (NP) Diploma in Biomedical Science (BMS) is a 3-year programme. In the first year, students attend all lectures at the NP campus. For second and third years students experience modular rotation to: a) NUH Department of Laboratory Medicine, b) a regional acute care general hospital (Tan Tock Seng Hospital), and c) small group attachment to primary care facilities throughout Singapore (polyclinics). Apart

from the core subjects, modules taught include basic and advance topics on instrumentation, chemistry, haematology, and microbiology. In addition, there are modules on molecular diagnosis, laboratory administrative management (material management, laboratory information system, financial budgeting), and a certification programme in phlebotomy. At NUH, the undergraduates attend lectures, tutorials and laboratory hands-on. Clinical professional staff and medical technologists teach subjects in chemistry, haematology, microbiology, molecular diagnosis, phlebotomy, and laboratory management. Staff on bench teach theoretical and laboratory skills. Overall the course aims to provide a rounded education where opportunities in sports and outdoor activities are contained in the curriculum.

A total of 228 students have enrolled for the programme with a ratio of 22% male to 78% female, and generally are 18 years (73%) of age in the 14-year analysis since inception of program.

Locally, the BMS diploma is well received. Generally the graduates are being offered jobs even before graduation. The advantage is that the graduates are functional from day one of employment. Internationally, the BMS programme is recognised in many countries. In the Australia and United Kingdom, students have been granted a direct entry into final year admission to universities for conversion to a Bachelor of Science Degree.

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Mechanisms of photodynamic therapy induced apoptosis in human skin

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Photodynamic therapy (PDT) is a non-invasive treatment for malignant and non-malignant skin conditions. It involves application of a photosensitiser followed by activation with visible light. The resulting photochemical reaction produces a cascade of events culminating in cell death. Apoptosis is believed to have an important role in the PDT response. In addition, production of TNF-alpha may enhance the therapeutic efficacy, contributing to both apoptosis and inflammation. We have made an immunohistochemical study of the apoptotic response to PDT in human skin.

Seven healthy volunteers participated in the study. Topical PDT was performed on buttock skin using 20% 5-aminolevulinic acid (ALA) cream followed by exposure to red light. Punch biopsies were taken 1, 4 and 24

hours post PDT and from untreated contralateral buttock skin.

On histological assessment, epidermal sunburn cells were significantly increased after 1 hour and were maximal after 24 hours (baseline: $0.2 \pm SE 0.1$ cells/hpf; 1 hour: 1.5 ± 0.5 , $p=0.05$; 24 hours: 1.9 ± 0.5 , $p=0.01$). The TUNEL assay revealed a significant increase in the number of apoptotic cells 4 hours post PDT with the increase being maintained after 24 hours (baseline: $163.5 \pm SE 31$ cells/hpf; 1 hour: 180.3 ± 7 ; 4 hour: 203.4 ± 26 , $p=0.02$; 24 hours: 194 ± 14). Caspase-3 protein expression was increased significantly at 1 and 4

hours post PDT (baseline: $18.2 \pm SE 7$ cells/hpf; 1 hour: 21.5 ± 6 , $p=0.03$; 4 hour: 29.7 ± 10 , $p=0.04$; 24 hour: 26.56 ± 6). No change was observed in expression of the anti-apoptotic protein Bcl-2. Significant up-regulation was observed in the expression of TNF-alpha from a baseline of $54.8 \pm SE 27$ cells/hpf to 92.4 ± 41.3 4 hours post PDT ($p=0.05$).

Hence apoptosis, involving activation of caspase-3, is an early event following PDT in human skin. Although the anti-apoptotic factor Bcl-2 appears not to be involved, the potential role of TNF-alpha is highlighted for further study.