Supplement

Proceedings of the Australasian Association of Clinical Biochemists’ 53rd Annual Scientific Conference

<table>
<thead>
<tr>
<th>INVITED SPEAKERS</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 HBA1C FOR DIAGNOSIS (INCLUDING PREGNANCY AND IN CHILDREN)</td>
<td>S11</td>
</tr>
<tr>
<td>A McElduff</td>
<td></td>
</tr>
<tr>
<td>S2 IMPLEMENTATION OF HBA1C AS A DIAGNOSTIC TEST IN NEW ZEALAND</td>
<td>S11</td>
</tr>
<tr>
<td>CM Florkowski</td>
<td></td>
</tr>
<tr>
<td>S3 THE INTRODUCTION OF HBA1C AS A DIAGNOSTIC TEST IN AUSTRALIA</td>
<td>S11</td>
</tr>
<tr>
<td>G Jones</td>
<td></td>
</tr>
<tr>
<td>S4 TIME TO RETIRE MICROALBUMIN? – NEGATIVE</td>
<td></td>
</tr>
<tr>
<td>G Jones</td>
<td></td>
</tr>
<tr>
<td>S5 TIME TO RETIRE MICROALBUMIN? - AFFIRMATIVE</td>
<td>S11</td>
</tr>
<tr>
<td>R MacIssac</td>
<td></td>
</tr>
<tr>
<td>S6 BIOCHEMISTRY OF PHAEOCHROMOCYTOMA</td>
<td>S12</td>
</tr>
<tr>
<td>G Eisenhofer</td>
<td></td>
</tr>
<tr>
<td>S7 LABORATORY TESTING FOR PRIMARY ALDOSTERONISM IN 2015 – CURRENT SITUATION AND IMPORTANT NEW DEVELOPMENTS</td>
<td>S12</td>
</tr>
<tr>
<td>M Stowasser</td>
<td></td>
</tr>
<tr>
<td>S8 THE PERFORMANCE/ADEQUACY OF CURRENT LABORATORY ASSAYS FOR GH AND IGF1</td>
<td>S12</td>
</tr>
<tr>
<td>KKY Ho</td>
<td></td>
</tr>
<tr>
<td>S9 GETTING THE RIGHT ANSWER – THE IMPORTANCE OF TRACEABILITY</td>
<td>S12</td>
</tr>
<tr>
<td>G Jones</td>
<td></td>
</tr>
<tr>
<td>S10 REVIEW OF QAP ACTIVITIES</td>
<td>S13</td>
</tr>
<tr>
<td>K Barancek</td>
<td></td>
</tr>
<tr>
<td>S11 CLOSING PLENARY – HYPOTHETICAL</td>
<td></td>
</tr>
<tr>
<td>T Badrick</td>
<td></td>
</tr>
<tr>
<td>S12 DIABETIC HYPERLIPIDAEMIA</td>
<td>S13</td>
</tr>
<tr>
<td>D Sullivan</td>
<td></td>
</tr>
<tr>
<td>S13 FIBROGENESIS BIOMARKERS IN DIABETES COMPLICATIONS</td>
<td>S13</td>
</tr>
<tr>
<td>SM Twigg</td>
<td></td>
</tr>
<tr>
<td>S14 ADVANCED GLYCATION IN DIABETES AND EFFECT OF DIET</td>
<td>S13</td>
</tr>
<tr>
<td>J Forbes</td>
<td></td>
</tr>
<tr>
<td>S15 INSULIN ASSAYS FIT FOR PURPOSE?</td>
<td>S13</td>
</tr>
<tr>
<td>P Williams, N Pereria, MS Twigg, T Yen</td>
<td></td>
</tr>
<tr>
<td>S16 MECHANISMS OF β-CELL FAILURE IN TYPE 2 DIABETES</td>
<td>S14</td>
</tr>
<tr>
<td>R Laybutt</td>
<td></td>
</tr>
<tr>
<td>S17 CLOSING THE LOOP OR RUNNING IN CIRCLES? MAKING PROGRESS</td>
<td>S14</td>
</tr>
<tr>
<td>J Gunton</td>
<td></td>
</tr>
<tr>
<td>S18</td>
<td>ASSESSMENT OF POLYCYSTIC OVARY SYNDROME</td>
</tr>
<tr>
<td>S19</td>
<td>FGF-21 AS A METABOLIC HORMONE</td>
</tr>
<tr>
<td>S20</td>
<td>BMAL AND THE METABOLIC CLOCK</td>
</tr>
<tr>
<td>S21</td>
<td>STEROID ANALYSIS BY LC-MS</td>
</tr>
<tr>
<td>S22</td>
<td>MACROPROLACTIN – CURRENT PROCESSES AND RECOMMENDATIONS</td>
</tr>
<tr>
<td>S23</td>
<td>ASSAYS FOR MONITORING CUSHING SYNDROME</td>
</tr>
<tr>
<td>S24</td>
<td>CORTISOL RESPONSES CAN IDENTIFY INDIVIDUALS WITH INCREASED SUSCEPTIBILITY TO OBESITY</td>
</tr>
<tr>
<td>S25</td>
<td>MOLECULAR DIAGNOSTICS IN THYROID CANCER</td>
</tr>
<tr>
<td>S26</td>
<td>MOLECULAR TESTING IN PHAEOCHROMOCYTOMA</td>
</tr>
<tr>
<td>S28</td>
<td>QUALITY OF HBA1C MEASUREMENTS IN AUSTRALIA –A REPORT FROM THE RCPAQAP</td>
</tr>
<tr>
<td>S29</td>
<td>ENDOCRINE PROGRAM – A WORKING REVIEW</td>
</tr>
<tr>
<td>S30</td>
<td>BIOCHEMICAL ASSESSMENT OF “BONE HEALTH”</td>
</tr>
<tr>
<td>S31</td>
<td>HYPOPHOSPHATAEMIA</td>
</tr>
<tr>
<td>S32</td>
<td>CALCIUM-SENSING RECEPTORS: MAKING SENSE OF CALCIUM REGULATION</td>
</tr>
<tr>
<td>S33</td>
<td>ENDOCRINE PROGRAM ANALYTE UPDATE</td>
</tr>
<tr>
<td>S34</td>
<td>COMMON RESULTS: ARE WE GOOD ENOUGH YET?</td>
</tr>
<tr>
<td>S35</td>
<td>INTERPRETING EQA AND ASSAYS AT RISK</td>
</tr>
<tr>
<td>S36</td>
<td>LC-MS VITAMIN D - A GAME CHANGER FOR LABS</td>
</tr>
<tr>
<td>S37</td>
<td>HUMAN HEALTH AND VITAMIN D STATUS; ‘WHY VITAMIN D TRIALS AND META-ANALYSES OFTEN INDICATE NEGATIVE RESULTS’</td>
</tr>
<tr>
<td>S38</td>
<td>VITAMIN D: HYPE, HOPE AND REALITY</td>
</tr>
</tbody>
</table>
QAP CLINICAL CASES
K Barancek, G Jones, H Martin, L Nguyen, G Ward

ORAL PRESENTATIONS

A1 DRIED BLOOD SPOT 17 HYDROXYPROGESTERONE MEASUREMENT UTILISING ON-LINE SPE COUPLED TO UPLC-MS/MS
BC McWhinney, AJ Wilce, JPJ Ungerer

A2 PLASMA FREE METANEPHRINES BY LC-MSMS: COMPARISON OF METHODS IN USE ACROSS AUSTRALIA AND NEW ZEALAND
M Whiting, G Woolard, B McWhinney, A Ellis, K Hoad, T Novos, T Andersen, S Koetsier

A3 EVALUATION OF ABBOTT ARCHITECT URINE FREE CORTISOL CUT-OFF
G Rathnayake, I Bretherton, Q Lam, B McWhinney, M Grossmann, J Grant, M Harrop, H Schneider, M Mohr, C Chiang

A4 HORMONE MODELLING IN PRETERM NEONATES: ESTABLISHMENT OF SERUM STEROID REFERENCE INTERVALS BY LC-MS/MS
RF Greaves, J Pitkin, CS Ho, J Baglin, RW Hunt, MR Zacharin

B1 ANTIBODY INTERFERENCE IN ACTH IMMUNOASSAY COMPLICATING MANAGEMENT OF CUSHING’S DISEASE
KW Choy, J Teng, N Wijeratne, CY Tan, JCG Doery

B2 CORTICOSTEROID-BINDING GLOBULIN CLEAVAGE IS PARADOXICALLY REDUCED IN ALPHA-1 ANTITRYPSIN DEFICIENCY: IMPLICATIONS FOR CORTISOL HOMEOSTASIS
MA Nenke, M Holmes, W Rankin, JG Lewis, DJ Torpy

B3 LABORATORY MANAGEMENT OF HAEMOLYSED SAMPLES – RESULTS OF AN RCPAQAP SURVEY
P Petinos

B4 SUPPORTING GLUCOSE TESTING OVER 1 MILLION SQUARE KILOMETRES
R Tirimacco, P Simpson, P Cowley, L Siew, P Tideman

B5 ANALYSIS OF TROPONIN T DELTA CHANGES FOR THE DIAGNOSIS OF AMI
P Tideman, R Tirimacco, P Simpson, P Cowley, L Siew, N Berry

C1 MODIFIED ABBOTT C16000 URINE PROTEIN METHOD FOR PATIENTS WITH DILUTE URINE
R Cherrie, R Flatman, C Ison, G Ward, L Price

C2 MEASUREMENT UNCERTAINTY FOR THE CLINICAL LABORATORY – A REVISION OF THE CONCEPT
GRD Jones

C3 THE RELATIONSHIP BETWEEN ROUTINE VITAMIN B6 LEVELS AND NON-P5P TRANSAMINASE ASSAY ACTIVITY
KA Sikaris, C Trambas, T Yen, D Kanowski, L Price, M Freemantle, ZX Lu

C4 WHAT IS AN EQUIVOCAL SERUM TOTAL VITAMIN B12 CONCENTRATION?
ZX Lu, JV Warner, J Chang, JCG Doery, AR McNeil, KA Sikaris

C5 ASSESSING THE QUALITY OF LABORATORY MEASUREMENTS OF HORMONES IN THE 2015 RCPA QAP ENDOCRINE PROGRAM
V Vamathevan, J Murby, L Jolly, K Barancek, G Jones

POSTER PRESENTATIONS

P1 URINARY CATECHOLAMINES MEASUREMENT BY LC/MS/MS
AC Yeoh, P Staneshwar, BBA Tan
P2 Urinary Metanephrine by LC/MS/MS for Clinical Use
AC Yeoh, P Staneshwar, BB Tan

P3 New Cut-off Value of the Vidas CMV IgG Avidity Give Less Borderline Results
A Lufianati, Y Liau

P4 Multistage Fragmentation of 1α, 25(OH)2-Vitamin D3 Combined with Metabolite Profile by LC/MS/MS Utilising a Quadrupole Linear Ion Trap
BR Cooke, M Wright, C Hodgkins

P5 Steroid Analysis in Different Matrices Utilising Standardised On-line SPE Coupled to UPLC-MS/MS
BC McWhinney, AJ Wilce, JPJ Ungerer

P6 Improved Measurement of Plasma Free Metanephrines Utilising On-line SPE Coupled to UPLC-MS/MS
B Myers, BC McWhinney, JPJ Ungerer

P7 Validation of a Shorter Spin Time of BD Vacutainer PST Tubes for Urgent Specimens
C Woods, C Oakman, J Farquhar, P Hickman, D Hughes

P8 Routine Lipid Parameters in the Prediction of Small Dense LDL
KA Sikaris, C Trambas, N Taylor, G Watts, R Shirley, T Partridge, R Grant

P9 Evaluation of the Precision and Accuracy of HbA1c Assays
C Trambas, KA Sikaris, S Heathcote, R Woodburn, K Parfrey, E Maxwell, ZX Lu

P10 Paraprotein Interference in Automated Chemistry
SDC Thomas, R Lavery, J Wei

P11 Respinning Lithium Heparin Tubes After Refrigeration for Add on Requests
E Moore, J Estacio, J Farquhar, P Hickman, D Hughes

P12 i-Stat Troponin I – Sample Type Analysis and Comparison with Troponin T
FL Groeneveld, M Roser, GRD Jones

P13 Boric Acid Does Not Affect the Measurement of Urine Creatinine, Sodium, Potassium, Calcium, Chloride and Phosphate
FB Mohd Abu Bucker, SK Sethi, TP Loh

P14 Analytical Evaluation of the Roche Cobas b 101 HbA1c Assay
G Dimeski, K Bassett, J Johnston, N Brown

P15 Evaluation of Roche Cobas 6500 Urine Analyzer in a Diagnostic Laboratory
J Jabon, LZ Ong, S Saw, SK Sethi

P16 Case Report: Macro-Troponin T
GRD Jones, J Chung

P17 CSF Bilirubin Measurements on Roche Modular – Effect of Assay Reformulation
J Chung, GRD Jones

P18 Validation of Biochemical Testing of Fluid Samples by Dilution into a Serum Matrix: A Preliminary Investigation
J Chung, GRD Jones

P19 Performance Evaluation of C-Reactive Protein and Procalcitonin Test on the ichroma™ Smart Analyzer
J Yoo, JH Rim, HS Lim, JR Choi
P20 A NOVEL APPLICATION OF LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY TECHNOLOGY FOR ESTABLISHING THE PRESENCE OF TOPICAL STEROIDS IN A SKIN WHITENING CREAM
K Waller, A Hall, G Marshall, JV Warner

P21 N-ACETYL Cysteine interference in the Abbott and Cambridge Life Sciences Paracetamol Assays
L Nguyen, L Koc, D Cheshier

P22 LOW URINE pH CAN CAUSE FALSE-HIGH MEASUREMENT OF 5-HIAA BY LC-MSMS
M Whiting

P23 EVALUATION OF THE EFFECT OF ABNORMAL HAEMATOCRIT LEVELS UPON THE ACCURACY OF HOSPITAL GRADE BLOOD GLUCOSE METERS
P Talsma, P Hickman, J Potter

P24 EVALUATION OF CREATININE AND CHLORIDE ON THE EPOC BLOOD GAS AND ELECTROLYTE POINT OF CARE ANALYSER
R Tirimacco, PA Simpson, L Siew, P Cowley, N Velleman, PA Tideman

P25 CHANGE IN STATUS OF SUNSHINE VITAMIN WITH GENERAL AWARENESS: STILL LOTS TO BE DONE
S Kaur, S Puri, S Parminder, I Verma

P26 REVERSE PHASE COLUMN CHROMATOGRAPHY (RPCC) FOR HIGH SPEED SEPARATION OF AMINO ACIDS AND ITS DERIVATIVES WITHIN BASIC AND LIMITED RESOURCE REDUCED SETTINGS
V Lal, A Kumar, M Parakh

P27 WHICH SPECIMEN TUBE IS BEST FOR SERUM/PLASMA OR WHOLE BLOOD TRACE ELEMENT ANALYSIS?
R Bahnisch, J Clark, W Rankin, M Saleem

P28 USUAL PATTERN OF SERUM FREE THYROXINE DISTRIBUTION IN CHILDREN ON THYROXINE FOR HYPOTHYROIDISM
LK Chin, L Dentinos, KW Choy, JCG Doery, ZX Lu, P Bergman

P29 METROLOGICAL TRACEABILITY OF ARCHITECT ALKALINE PHOSPHATASE AND AMYLASE ASSAYS TO IFCC REFERENCE METHODS
D Armbruster, D Yahalom, L Lennartz, M Orth

P30 ASSESSING ANALYTICAL QUALITY OF Hb A1c ASSAYS USING REFERENCE SAMPLES, ACCURACY BASED GRADING, AND SIGMA METRICS
D Armbruster, G Maine

P31 LIPOPROTEIN(a) STATUS AND LDL-CHOLESTEROL IN TYPE 2 DIABETES MELLITUS WITH MICROVASCULAR COMPLICATIONS
KA Jhuma, ASM Giasuddin, AMM Haq

P32 OBESITY RELATED METABOLIC ABNORMALITIES IN A GROUP OF SRI LANKAN CHILDREN
L Ginige, D Samaranayake, MM Gunatillaka, VP Wickramasinghe

P33 FLAT ORAL GLUCOSE TOLERANCE TEST REVISITED
M M Salih, A Simpson, P E Hickman, S Apostoloska, C Yu, J M Potter

P34 BIO-RAD D100 HBA1C – THE SAME CHROMATOGRAPHY IN HALF THE TIME
R King, CR Hawes, CM Fiorkowski

P35 METFORMIN DELAYS PROGRESSION TO TYPE 2 DIABETES BY 60% – RESULTS OF TEN YEARS OF ROUTINE CLINICAL USE
SC Martin
P53 REPORTING SERUM CARCINOEMBRYONIC ANTIGEN (CEA) VALUES IN RECTAL CANCER PATIENTS RECEIVING ADJUVANT RADIOThERAPY BEFORE SURGERY: SPOTLIGHT ON THE UTILITY OF PATIENT DERIVED DATA
S Chakraborty, S Banerjee, I Mallick, S Sen, P Roy

P54 Fecal Calprotectin Testing Protocol for Diagnosis of Inflammatory Bowel Disease – A New Zealand Experience
S Hemmady, T Marcinkowski

P55 Characteristics of Individuals with Elevated Troponin I on a High Sensitivity Assay but Normal Levels on a Contemporary Assay
R Bender, F Njue, S Vasikaran, R Lambert, J Rankin, D Bell

P56 Outcome of Specific Interpretative Comments Suggesting Specialist Referral for Individuals at High Risk of Familial Hypercholesterolaemia
R Bender, G Edwards, J McMahon, AJ Hooper, GF Watts, JR Burnett, DA Bell

P57 Impact of IL-6 Gene on Male Infertility - Study on an Indian Populations Instead of “Association of IL-6 Gene with Male Infertility - Study on an Indian Populations
KK Shukla, P Sharma, AA Mahdi, SN Sankhwar

P58 Pro-(IL-18) and Anti-(IL-10) Promoter Genetic Variations Affects Susceptibility and Their Serum Levels in Prostate Carcinoma
S Dwivedi, P Sharma, A Goel, S Khatri, KK Pant

P59 Gene Expression of Apoptotic Pathway in Male Fertility
P Sharma, KK Shukla, S Chambia, SN Sankhwar

P60 Determination of a Reference Interval for IGF1 by Data Mining Using the Bhattacharya Technique
D Kanowski, B Teis, M Freemantle, L Price, G Ward

P61 17-Hydroxyprogesterone Assays – Time for Harmonisation
R Tudball, KL Wan, KW Chov, N Wijeratne, J Montalto, C Tran, L Jolly, JCG Doery

P62 A 17-Year Audit of Testing for 21-Hydroxylase Deficiency
KL Wan, Chov KW, N Wijeratne, JCG Doery

P63 Evaluation of Roche and Beckman Coulter Automated Anti-Mullerian Hormone Assays
M Freemantle, B Teis, K Young, G Ward, D Kanowski, L Price

P64 Development of a Method for the Simultaneous Measurement of Serum Testosterone, 17-Hydroxy Progesterone and Androstenedione by Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS)
M Henman, K Waller, J Warner

P65 Review of Sex Hormone Test Requests in a Tertiary Hospital
L Ong, S Saw, SK Sethi

P66 Prolactin and Reproductive Hormone Status in Oligomenorrheic and Infertile Females
R Suwal, AK Nepal, B Gelal, S Gautam, BKL Das, M Lamsal, S Majhi, N Baral

P67 Development of an Urine Cortisol Assay by Liquid Chromatography-Tandem Mass Spectrometry
R Sahertian, M Wright, P Stanford, C White

P68 Integrating LC-MS/MS into a Clinical Laboratory: Hands-Free Data Transfer From Sample Handling to Result Entry
R Sahertian, M Wright, B Patterson, C Hodgkins, J Teal, C White
P69 **A PRAGMATIC APPROACH TO PAEDIATRIC REFERENCE INTERVALS FOR CEREBROSPINAL FLUID (CSF) TOTAL PROTEIN, GLUCOSE AND WHITE BLOOD CELL COUNT USING PUBLISHED EVIDENCE, CLINICAL CONSENSUS AND IN-HOUSE VERIFICATION**

CKM Ho, NWS Tee

P70 **TRIMESTER SPECIFIC REFERENCE INTERVALS FOR THYROID HORMONES IN THE SINGAPORE POPULATION**

ETH Tan, CKM Ho, GSH Yeo, KYC Kwek, BSM Chern, KH Tan

P71 **THYROID FUNCTION TEST REFERENCE INTERVALS DURING PREGNANCY USING THE VITROS 5600**

E Haworth, C Chang, T Yen, A Chiriano, S Matthews

P72 **TOWARDS AGE-RELATED REFERENCE INTERVALS FOR THE HCG STIMULATION TEST IN CHILDREN WITH SUSPECTED DISORDERS OF ANDROGENISATION**

WK Choong, KW Choy, J Brown, P Bergman, JCG Doery

P73 **PAEDIATRIC REFERENCE INTERVALS FOR IGF1 AND IGFBP3 IN SHORT BUT GROWTH HORMONE-REPLETE CHILDREN**

JD Newman, KW Choy, TP Loh, JCG Doery

P74 **URINE PROTEIN CREATININE RATIO IN PREGNANCY – PERFORMANCE OF BENZATHONIUM CHLORIDE AND ENZYMATIC CREATININE**

P Ward, S Bandodkar, T Yen

P75 **EVALUATION OF NEWBORN SCREENING FOR THE DIAGNOSIS OF CARNITINE UPTAKE DEFICIENCY IN INDIAN POPULATION**

S Mohapatra, AC Poonima, R Devi, R Cariappa

P76 **IMPLEMENTATION OF PLACENTAL GROWTH FACTOR INTO FIRST TRIMESTER SCREENING FOR DOWN SYNDROME**

S Khouri, M Long, M Bonifacio

P77 **HIGH FREQUENCY OF UNDETECTABLE URINE PROTEIN DURING SCREENING FOR GESTATIONAL PROTEINURIA – TRUE LOW PROTEIN OR CLINICAL PROTEINURIA MASKED BY EXCESSIVELY DILUTE URINE?**

T Yen, E Haworth, A Chiriano, S Matthews

P78 **IN VITRO FERTILISATION (IVF) AND FIRST TRIMESTER SCREENING (FTS) FOR DOWN SYNDROME.**

R Sinnadurai, JM Morris, V Tasevski

P79 **A TRAINING AND COMPETENCY ASSESSMENT PROGRAM AT RCPAQAP**

P Petinos, F Intan

P80 **SHOULD WE TARGET SET OUR RCPAQAP MATERIAL WHEN THERE ARE GAPS IN THE TRACEABILITY CHAIN? TARGET ASSIGNMENT OF 17-OHP, ANDROSTENEDIONE, DHEA AND DHT BY GC-MS/MS**

RF Greaves, L Jolly, MF Hartmann, SA Wudy

P81 **ESTABLISHMENT OF AN EXTERNAL QUALITY ASSURANCE PROGRAM FOR SERUM DIHYDROTESTOSTERONE**

L Jolly, SA Wudy, MF Hartmann, CS Ho, RKT Kam, J Joseph, C Boyder, RF Greaves

P82 **UNDERSTANDING QC, EQA AND METHOD EVALUATION - A PROGRESSIVE APPROACH TO PREPARING WORK-READY GRADUATES**

FN Breen, SL Weier

P83 **A SIMPLE TOOL TO IDENTIFY AT RISK ASSAYS USING EQA DATA**

M Mackay, G Hegedus, TC Badrick

P84 **END OF AN ERA – DECOMMISSIONING THE ABBOTT TDxFLx FOLLOWING THE ASSESSMENT OF TWO CHEMILUMINESCENT METHOTREXATE IMMUNOASSAYS**

A Chiriano, C Tran, E Haworth, S Matthews
HOW LOW CAN YOU GO? AN EVALUATION OF THE NEW ABBOTT ARCHITECT ci2000 IMMUNOASSAY IN THE THERAPEUTIC DRUG MONITORING OF METHOTREXATE
HM Pawson

RAPID AND EFFICIENT ENZYMATIC HYDROLYSIS OF CODEINE AND MORPHINE GLUCURONIDES IN URINE
K Ellis, W McConnell, M Huynh, S Sacks, J Grasko, L Dusci

AN EVALUATION OF THE ABBOTT ARCHITECT ASSAY AGAINST THE ABBOTT TDX METHOTREXATE ASSAY
M Al-Hinti, P Williams, E Lin

LC-MSMS ALLOWS DETECTION AND MONITORING OF ANTI-PSYCHOTIC POLYPHARMACY
MJ Whiting

LABORATORY EVALUATION OF FOUR DRUGS OF ABUSE IMMUNOASSAY KITS
JW Lo, CA Quinlan, W McConnell, S Sacks, J Grasko
S1 HBA1C FOR DIAGNOSIS (INCLUDING PREGNANCY AND IN CHILDREN)
A McElduff
Discipline of Medicine, The University of Sydney, Sydney, NSW, Australia
Glycated haemoglobin reflects the average plasma glucose concentration over the prior 8-12 weeks. It quantifies the risk of macrovascular disease and of the microvascular complications of diabetes mellitus. A number of medical (and technical) issues can confound these relationships. The World Health Organization (WHO) conducted a systematic review of the use of HbA1c as a diagnostic test for diabetes mellitus. An HbA1c of 6.5% was recommended as the cut point for diagnosing diabetes. A value <6.5% does not exclude diabetes diagnosed using glucose tests. The WHO expert group concluded that there was currently insufficient evidence to make any formal recommendation on the interpretation of HbA1c levels below 6.5%.

The WHO now recognises two levels of hyperglycaemia first detected in pregnancy: diabetes mellitus in pregnancy and gestational diabetes. In late pregnancy, for minor degrees of hyperglycaemia (gestational diabetes), glycated haemoglobin has been shown to be less effective in predicting adverse pregnancy outcomes than the traditional 75 g OGTT (Diabetes Care 2012;35:574). However, a HbA1c >6.5% would still indicate diabetes mellitus in pregnancy with the same caveat about values <6.5% outside of pregnancy. Work from NZ suggests an early pregnancy HbA1c ≥5.9% is optimal for detecting diabetes and identifies women at increased risk of adverse pregnancy outcomes (Diabetes Care 2014;37:2953).

There are almost no published data on this issue in children. In NSW, type 2 diabetes is about 10% of diabetes in adolescence. Type 2 diabetes is rare before puberty. Most diabetes in children is diagnosed clinically on symptoms and an appropriately elevated plasma glucose level. The international paediatric guidelines suggests that glycated haemoglobin may be useful as a diagnostic aid in borderline situations or in incidentally discovered mild hyperglycaemia in an asymptomatic child. The test is not recommended for routine use (Pediatric Diabetes 2014;15(Suppl. 20): 4–17).

S2 IMPLEMENTATION OF HBA1C AS A DIAGNOSTIC TEST IN NEW ZEALAND
CM Florkowski
Canterbury Health Laboratories, Christchurch, New Zealand
In 2003, the consultation process in New Zealand dates back to 2003, well before the international recommendations were made. It reflects the close cooperation between the clinical and laboratory communities in New Zealand, particularly through the agency of the New Zealand Society for the Study of Diabetes (NZSSD), a key organisation in New Zealand open to all those involved in the care of people with diabetes and the national advisory body on scientific and clinical diabetes care and standards. There was a phased process of consultation designed to increase familiarity and comfort with the new units and the final step was coupled with the adoption of HbA1c as a diagnostic test with some evidence-based pragmatism around using the rounded cut-off. Key clinicians were committed to the new units from an early stage. Genuine clinical engagement is vital in such a process. (Clin Chim Acta 2014;432:157–61).

S3 THE INTRODUCTION OF HBA1C AS A DIAGNOSTIC TEST IN AUSTRALIA
G Jones
Chemical Pathology, St Vincent’s Hospital, Sydney, NSW, Australia
Prior to 2012, diabetes diagnosis in Australia was based solely on plasma glucose measurements. Following recommendations from the World Health Organization in 2011, consideration was commenced on the use of HbA1c for diagnosis of diabetes in Australia. The process has largely been threefold. Firstly a formal statement that HbA1c could be used for diagnosis. This was published in the Medical Journal of Australia in August 2012. The second in the inclusion of HbA1c for diagnosis on the Medicare Schedule of Benefits which occurred in November 2014. The third important factor is education on the most appropriate way to use this test. This last item remains an ongoing process with the need for requesting doctors to order the test is both the most suitable clinical manner as well as in keeping with Medicare rules. By April 2015 the number of HbA1c tests being funded by Medicare for diagnosis remains less than one quarter the number being used for diagnosis. This suggests that there is room for improvement on education on the best use of this test for this indication.

S5 TIME TO RETIRE MICROALBUMIN? - AFFIRMATIVE
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The concept of microalbuminuria has been central to the development of clinical practice and research in the area of diabetic kidney disease (DKD). However, in recent times, the value of a paradigm of DKD based solely on microalbuminuria has been questioned. Although both the absolute level and rate of change of microalbuminuria are linked to the development and progression of DKD, microalbuminuria on its own lacks the necessary sensitivity or specificity to accurately predict kidney outcomes for people with diabetes. The development of microalbuminuria can no longer be viewed as a committed and irreversible stage of DKD as spontaneous remission is now reported as a common occurrence. In addition, the absence of microalbuminuria or its progression to proteinuria does not signify that an individual patient is safe from a progressive decline in glomerular filtration rate (GFR). Furthermore, although reductions in albuminuria within the microalbuminuric range can be linked to a slower GFR decline in observational studies, this relationship has not been robustly demonstrated in intervention studies. Conclusions regarding the kidney health of individuals with diabetes will continue to be flawed if an inappropriate emphasis is placed on the presence or absence of albuminuria or changes in albuminuria within the microalbuminuric range. This has important implications in terms of undermining the value of microalbuminuria as a surrogate renal endpoint for intervention trials. There is also a need to develop broader models of progressive DKD that include novel pathways and risk markers apart from those related to the traditional ‘albuminuric-pathway’ to renal impairment. At the very least, it is time to retire the term ‘microalbuminuria’ even if low levels of albuminuria are still interpreted to signify kidney damage. Indeed, most guidelines now actively discourage the use of the term ‘microalbuminuria’ and instead suggest assigning albuminuria to three categories: A1, normal to mildly elevated; A2, moderately increased (instead of microalbuminuria); and A3, severely increased (instead of macroalbuminuria or proteinuria).

S6 BIOCHEMISTRY OF PHAEOCHROMOCYTOMA
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Phaeochromocytomas are catecholamine-producing and metabolising tumours of adrenal chromaffin cells. Paragangliomas are closely related tumours arising from extra-adrenal chromaffin cells. The highly heterogeneous nature of chromaffin cell tumours reflects the diversity of underlying germline and somatic mutations. Differences in proportions and amounts of the three catecholamines – dopamine, noradrenaline and adrenaline – synthesized, stored, metabolized and secreted by the tumours varies depending on underlying mutations, which also impact other disease presentations. Based on transcriptomic profiles, the tumours fall into two main groups: those due to mutations leading to stabilization of hypoxia-inducible factors (HIFs) and activation of hypoxia-angiogenic pathways (cluster 1) and those due to mutations impacting kinase signalling pathways (cluster 2). The latter are well differentiated, almost always at adrenal locations, rarely malignant and characterised by extensive vascular stores of both adrenomedullary and noradrenaline. These tumours have low rate constants of catecholamine secretion, reflecting well-developed exocytotic pathways, resulting in often normal or negligibly increased plasma or urinary catecholamines, but consistently increased levels of both metanephrine and normetanephrine. In contrast, cluster 1 tumours are more immature, occur at an earlier age and more often develop at extra-adrenal locations compared to cluster 2 tumours. Even when present at adrenal locations, these tumours do not produce significant amounts of adrenaline, a result of HIF2α-mediated blockade of steroid induction of phenylethanolamine-N-methyltransferase. These tumours are therefore diagnosed from solitary increases in plasma or urinary normetanephrine. They have low tissue stores of noradrenaline, which are nevertheless released at continuously high rates due to relative lack of secretory controls. Among cluster 1 tumours due to mutations affecting the Krebs cycle, there is additional epigenetic silencing of genes coding for components of catecholamine biosynthetic and secretory pathways. These phenotypically immature tumours are prone to malignancy and often make significant quantities dopamine, best assessed from measurements of plasma methoxytyramine.

PLENARY 4

S7 LABORATORY TESTING FOR PRIMARY ALDOSTERONISM IN 2015 – CURRENT SITUATION AND IMPORTANT NEW DEVELOPMENTS

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Primary aldosteronism (PA) is a common cause of hypertension and associated with morbidity excessive for degree of hypertension and reduced quality of life, all reversible with specific surgical or medical treatment. Optimal detection requires accurate yet readily applicable diagnostic approaches and awareness of factors that may confound results. In addition to previously recognized confounders (including antihypertensive medications, posture, time of day, dietary salt intake and plasma potassium) of the plasma aldosterone/renin ratio (ARR), recent studies have drawn attention to effects of gender, and the potential for false positives during the luteal phase of the menstrual cycle and in women receiving oestrogen-containing contraceptive agents when direct renin concentration (but not plasma renin activity) is used to calculate the ARR. Selective serotonin reuptake inhibitor antidepressants lower the ARR (potential for false negatives). Fludrocortisone suppression testing, probably the most reliable means of definitively confirming or excluding PA, is time consuming, cumbersome and expensive, requiring a five day inpatient stay. A new approach, upright (seated) saline infusion suppression testing (SST), has shown excellent reliability in a recent pilot study, with much greater sensitivity than conventional recumbent SST, and requiring only a morning outpatient visit. Differentiation of unilateral (surgically correctable) from bilateral (usually treated medically) forms of PA is essential for optimal management selection. The most reliable approach, adrenal venous sampling (AVS) requires considerable expertise. Optimisation of AVS cannulation success rates requires experience and high throughput (assisted by limiting the number of radiologists performing AVS in each institution) and can be further enhanced by using computed tomography to localise the adrenal veins and point-of-care cortisol testing. Assay reliability is essential for accurate PA workup, and the introduction into clinical practice of highly reliable, high-throughput mass spectrometric (MS) methods of measuring aldosterone has represented a major advance. MS approaches to assessing renin/angiotensin activity are in development.

S8 THE PERFORMANCE/ADEQUACY OF CURRENT LABORATORY ASSAYS FOR GH AND IGF1

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Immunoassays are the backbone of the practice of endocrinology. Immunoassays measuring circulating concentrations of growth hormone (GH) insulin-like growth factor 1 (IGF-I) are used to diagnose and manage GH-related diseases such as GH deficiency and acromegaly. Commercial assays are routinely employed in almost all laboratories nationally and internationally. The diagnostic utility for GH is defined through peak or nadir concentrations in response to dynamic tests and for IGF-I by references ranges supplied by the manufacturer. The various immunoassays employ different standards, calibrators, antibodies, reagents and procedures. The heterogeneity has resulted in variability between assay readouts that can exceed 200%, limiting the application of consensus guidelines in clinical practice. The units of expression (international units vs mass units) can differ between assays and require the use of conversion factors, adding to the uncertainty and confusion around interpretation. Reference ranges, essential for identifying disease and guiding therapy, are affected by sample size and biological factors such as age, gender and nutrition. The information on references ranges in most commercial assays is poor with most not providing adult age-stratified values which limits clinical utility and may misguide therapeutic decision making. The performance and utility of current GH and IGF-I assays are unsatisfactory. International workshops have proposed measures to improve the comparability of assay results. This includes the use of a commutable standards and the reporting only in mass units as first steps in assay harmonisation. National and International professional bodies overseeing measurement standards and their application have an important role which includes seeking cooperative involvement from industry.

PLENARY 5 – QA UPDATE

APFCB TRAVELLING LECTURE

S9 GETTING THE RIGHT ANSWER – THE IMPORTANCE OF TRACEABILITY

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Results of laboratory tests are used for medical decision-making with the aim of improving patient health. A fundamental part of the role of the routine laboratory is getting the right result for a laboratory test. This means having assay with good precision, low bias and freedom from interferences. Bias in particular can affect all results for a test leading to incorrect decisions on some patients. Assay results are derived by comparing values in patient samples with values in the assay calibrators. The values for concentrations in calibrators are set by comparison with other calibrators. As we compare laboratory results with decision points and information in the medical literature from all parts of the world, ideally we need to ensure that all results for a test are comparable across the globe. One part of this is selecting the international reference materials and methods that manufacturers should use for setting calibrator values. The Joint Committee for Traceability in Laboratory Medicine (JCTLM) is a joining of international organisations in measurement and laboratory medicine to help with this task. Manufacturers should use the best available materials and methods to assign values to calibrators and laboratories should select traceable methods and verify accuracy to ensure the best outcome for patients.
S10 REVIEW OF QAP ACTIVITIES
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The RCPAQAP Chemical Pathology Program is a worldwide leader in the provision of external quality assurance programs offering 49 individual programs to over 2300 participants in 40 different countries. The RCPAQAP Chemical Pathology has seen significant changes over the last few years, most notably in the delivery of electronic reports to participants and the introduction of the myQAP customer portal. Our goal is to make your experience with the RCPAQAP even more efficient and convenient. This Review of Activities will focus on program changes and activities over the last 12 months and any new program initiatives planned for 2016. This session will also look at how the introduction of the myQAP has changed the way participants interact with the Chemical Pathology QAP.

SYMPOSIUM 1 - COMPLICATIONS

S12 DIABETIC HYPERLIPIDAEMIA
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Diabetes is a maladaptive alteration in macronutrient metabolism. It affects not only glucose and carbohydrate metabolism, but also the other major class of macronutrient class, namely lipids. Lipid abnormalities manifest as disturbances of the levels of the lipoproteins that transport lipids in the bloodstream. These disturbances may contribute towards the macrovascular complications of diabetes by influencing the processes that underlie atherosclerosis and thrombosis. Increased energy intake and reduction in activity in affluent and developing societies has resulted in an increase in the prevalence of type 2 diabetes. Clinical evaluation of lipoprotein metabolism in diabetes involves the measurement of total cholesterol, HDL-C and TG following a 12-hour fast. LDL-C is calculated by the Friedewald equation, but this calculation becomes less reliable as TG levels increase beyond 4 mmol/L. Non-fasting samples have been shown to be a more sensitive marker for the detection of individuals with increased risk of CVD, but the unstandardised nature of non-fasting samples makes them unsuitable for the serial monitoring of lipid status in diabetes. Variations in LDL particle composition complicate the relationship between LDL-C and CVD risk, leading to growing enthusiasm for other risk markers such as non-HDL-C or apolipoprotein B, particularly in the presence of elevated levels of TG.

The fundamental differences in the pathophysiology and treatment of type 1 and type 2 diabetes are also manifest in their accompanying changes in lipoprotein metabolism and its contribution towards atherosclerosis and macrovascular disease. Fully treated type 1 diabetes usually causes minimal disturbance in the lipoprotein profile, but the atherogenicity of the diabetic state in type 1, combined with the early age of onset, results in an increased life-long risk of CAD that demands efforts to maintain lipoproteins at target levels. Type 2 diabetes typically causes mild hypertriglyceridaemia with low HDL and small, dense LDL. If triglyceride removal becomes saturated, this picture may rapidly progress to massive hypertriglyceridaemia with associated risk of pancreatitis.

S13 FIBROGENESIS BIOMARKERS IN DIABETES COMPLICATIONS
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Diabetes mellitus is characterised by a lack of insulin causing elevated blood glucose, often with associated insulin resistance. Over time, especially in genetically susceptible individuals, such chronic hyperglycaemia can cause tissue injury. A major pathological response to tissue injury is the development of fibrosis, which involves predominant extracellular matrix (ECM) accumulation, especially in certain solid organs such as the kidney, heart and liver, and which may associate with organ functional loss. The main factors that regulate ECM in diabetes are thought to be pro-sclerotic cytokines and protease/anti-protease systems. This review will examine some identified factors and regulators of tissue fibrosis in diabetes and whether their levels in biological fluids may have clinical utility as clinical biomarkers of diabetes complications onset, progression, or protection. Examples in diabetes and retinopathy, CVD, and diabetes and foot ulceration will be particularly explored.

S14 ADVANCED GLYCATION IN DIABETES AND EFFECT OF DIET
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Advanced glycation end products (AGEs) are formed in the body when lysine and arginine residues in proteins and peptides become irreversibly modified by reactive sugars or carbohydrates. AGEs can also be absorbed from dietary sources, in particular from westernised diets as a result of modern food processing, storage and choice of cooking method. AGEs are arguably best studied in diabetes complications where increased burden of AGEs in the body, measured at sites such as the skin and circulation, can predict the later onset of diabetic complications. Recently, however, there has been a paradigm shift which suggests that AGEs, including those from dietary sources may be direct modulators of insulin secretion and peripheral insulin sensitivity and as such, may play a crucial role in the development of both major forms of diabetes per se. There is also evidence for kidney damage as a direct result of AGEs, independent of glycaemic control, which may also influence mortality. The major effects of are most likely via ligation with receptors such as the receptor for advanced glycation end products, RAGE but also with another relatively novel AGE receptor, AGE-R1. The signalling of AGEs via receptors is known to influence inflammatory and immune cascades and metabolic pathways involved cell growth and energetics.

SYMPOSIUM 2 - MECHANISMS

S15 INSULIN ASSAYS FIT FOR PURPOSE?
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Insulin assays crucial in distinguishing between type 1 and type 2 diabetes were described in 1950’s by Australian Prof. Bornstein. This was confirmed in the late 50’s when Berson and Yallow described the first immunoassay using patient and Guinea Pigs. This earned a Nobel Prize and was the first immunosassay. Insulin assays have been an essential component in assessing insulin secretion and peripheral insulin sensitivity and as such, has been achieved. An International Insulin standard provides a basis to convert insulin from the bioassay based IU/L to mass units (conversion factor 6) but despite being a homogenous hormone assays are still not been achieved. There a big differences in assay specificity to the new synthetic engineered insulin from the bioassay based IU/L to mass units (conversion factor 6) but many assays have different standards and different conversion factors. Dr. Sue Manly in 2007 (Clin. Chem 53:5, 922) drew attention to the difference between assay results and found a two-fold difference across them. This is a problem when people compare studies of insulin resistance using different insulin assays, haemolysis is a major problem for the assay, as are insulin antibodies. There a big differences in assay specificity to the new synthetic engineered insulin preparations. Most insulin assays can measure human insulin to some degree but not all commercial insulins. We have used the Architect assay to examine the changes in commercial insulin stored under various conditions to determine if they deteriorate under conditions that patients may travel or store insulin. Some Insulin assays allow coroners to determine causes of death from insulin abuse and the glucose-insulin ratio at 1 hr in the OGTT may predict those patients with secretory deficiency (presented at an earlier AACB meeting). Despite being a homogenous hormone assays are still not standardised which presents presents a challenge in laboratory and clinical practice.
S16 MECHANISMS OF β-CELL FAILURE IN TYPE 2 DIABETES
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The critical contribution of deficient insulin secretion to the pathogenesis of type 2 diabetes is beyond doubt. The normal β-cell response to obesity-associated insulin resistance is hypersecretion of insulin that maintains blood glucose levels within the normal range. This is associated with both expansion of β-cell mass and enhanced β-cell function. Type 2 diabetes only develops in subjects that are unable to sustain the β-cell compensatory response. This is associated with a progressive deterioration of β-cell function, particularly impairment of glucose stimulated insulin secretion (GSIS), and a loss of β-cell mass through an increased rate of apoptosis. Thus, type 2 diabetes arises in subjects with islets that are susceptible to dysfunction and apoptosis under conditions of high demand. Stress within the endoplasmic reticulum (ER) organelle of the cell has been proposed as a mechanism for β-cell dysfunction and death in type 2 diabetes. ER stress activates a signalling cascade known as the unfolded protein response (UPR) – the role of which is both to alleviate the ER stress through the upregulation of protein folding enzymes and chaperones and, paradoxically, to activate apoptosis via deleterious UPR signalling if the stress is too severe or prolonged. Recent findings suggest that upregulation of the adaptive UPR is linked with β-cell compensation and protection against obesity-associated diabetes. Conversely, in genetically susceptible β-cells, suppression of ER adaptation and loss of β-cell differentiation underlies β-cell failure and progression to diabetes. Factors leading to failure of ER adaptation include chronic hyperglycaemia, inflammation and hypoxia. This knowledge is critically important to understanding the mechanisms responsible for the switch from β-cell compensation to failure in type 2 diabetes.

S17 CLOSING THE LOOP OR RUNNING IN CIRCLES? MAKING PROGRESS
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Introduction
Curing a large proportion of people with Type 1 diabetes with β-cell replacement therapy remains a medium to long-term goal. Increasingly, it appears that closed-loop therapy where glucose sensing technology communicating with an insulin pump will be achievable for patients before β-cell replacement therapy. This requires intensive computational algorithms, and there are many different proposed approaches. Some investigators also recommend dual infusion with insulin and glucagon.

Conclusion
The combination of an insulin pump and CGMS (continuous glucose monitoring system) use is expensive, even if the CGMS is used intermittently. There are statistically and clinically significant changes in hypoglycaemia rates and in some studies HbA1c with this technology at its current level. The algorithms are improving, and this may become state of the art for those who can afford it in the near future.

SYMPOSIUM 3 - CLINICAL

S18 ASSESSMENT OF POLYCYSTIC OVARY SYNDROME
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PCOS is one of the most common conditions in Australian women, affecting a striking 12 to 21% of reproductive aged women depending on the study population and diagnostic criteria applied. PCOS has significant and diverse clinical implications including reproductive (hyperandrogenism, hirsutism, oligo/anovulation, subfertility), metabolic (metabolic syndrome, insulin resistance, impaired glucose tolerance (IGT), type 2 diabetes mellitus (T2DM), adverse cardiovascular risk profiles and possible increased cardiovascular disease) and psychological features (increased anxiety, depression, body image issues and worsened quality of life). PCOS is essentially a hormonal disorder underpinned by insulin resistance and hyperandrogenism. Obesity is common in PCOS and increases the prevalence and severity of the condition by further exacerbating IR.

PCOS is a heterogeneous condition and there is no single test to diagnose PCOS. Changing definitions and a variety of symptoms have made the path to diagnosis for many women difficult. It is estimated that up to 70% of women with PCOS in the community with PCOS remain undiagnosed. Diagnosis of PCOS is now recommended using the ‘Rotterdam’ criteria and is based on the presence of two of the following three criteria: hyperandrogenism (clinical or biochemical), oligomenorrhea / amenorrhoea or polycystic ovaries on ultrasound, with exclusion of related reproductive disorders. Insulin resistance is common in PCOS, but is not required for diagnosis, in part because of the lack of accurate methods to measure insulin resistance. Anti Mullerian Hormone (AMH) levels may be elevated in women with PCOS, but this is not currently included in the diagnostic criteria either. PCOS is increasingly being recognised as a metabolic disorder and women with PCOS should undergo regular screening for metabolic complications including impaired glucose tolerance and type 2 diabetes.

S21 STEROID ANALYSIS BY LC-MS
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The introduction and expansion of automated immunoassays in clinical chemistry and endocrinology had the advantage of increasing the number and diversity of assays and the speed at which they could be performed and results released. Economies of scale and structural efficiencies created the opportunity to incorporate and commercialise the sector through advances in automation and IT. Without trying to sacrifice analytical rigor the business model has shifted toward high throughput non-chromatographed and unseparated specimen analyses at reduced cost. However immunometric analysis of steroids has always had issues around sensitivity, and more importantly specificity, which can leave the clinician with the un-enviable question of “what is the true value of this test result?”. Liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) has the potential to provide a solution to this problem but there are many additional considerations to be taken into account before moving to this technology. This presentation will briefly cover immunoassay and LC-MS/MS techniques, why immunoassays struggle to measure steroids & how regulatory bodies may be a part of the problem rather than the cure, when LC-MS/MS can provide the answer but also the difficulties in knowing what to do with the LC-MS/MS result and situations where the correct answer isn’t always what we had hoped for.

S22 MACROPROLACTIN – CURRENT PROCESSES AND RECOMMENDATIONS
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Macroprolactin, a high molecular weight form of prolactin generally composed of prolactin bound to IgG, was first described over thirty years ago. Although it is biologically inactive macroprolactin is detected by most assays and is an ongoing problem. It is the role of the laboratory to provide a clinically meaningful prolactin level in the presence of macroprolactin.

Most laboratories now screen samples with a high prolactin level for the presence of macroprolactin using the polyethyleneglycol (PEG) procedure.
PEG precipitates immunoglobulin including macroprolactin. A low recovery of prolactin in the supernatant (free, bioactive or monomer prolactin) indicates that most prolactin is in the macroprolactin form and that the prolactin level is falsely elevated. Traditionally laboratories have reported only the pre-PEG prolactin level and presence or absence of macroprolactin generally with a comment that the prolactin was elevated due to macroprolactin if appropriate. As true hyperprolactinaemia can still exist in addition to macroprolactinaemia it is now recommended that the free or monomer prolactin level should be reported with appropriate reference intervals (eg determined using PEG treated specimens).

While reporting the free prolactin level provides a clinical meaningful measurement of prolactin a recent survey of Australian and New Zealand laboratories indicated that there is considerable variability in every step of the procedure, from what constitutes a high prolactin level to the details of PEG procedure and results reporting. There is certainly a need for standardisation of the procedure including reporting. Introduction of a macroprolactin external quality assurance program should assist in the harmonisation of results.

**SYMPOSIUM 5 – MOLECULAR ADVANCES**

**S25 MOLECULAR DIAGNOSTICS IN THYROID CANCER**

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Thyroid cancer is diagnosed by cytology of fine needle aspirates (FNA) from thyroid nodules. The cytology results are then classified using the Bethesda scale I-VI, where Bethesda II is benign and Bethesda VI is malignant. The Bethesda III-V categories are classified as undetermined significance to suspicious for malignancy and it is in these intermediate classifications where molecular diagnostic tools could be of the most benefit.

It is well recognised that the different types and variants of thyroid cancer are associated with particular DNA mutations. The current molecular analysis uses samples obtained through FNA and formalin fixed paraffin embedded (FFPE) tissue from surgery. FNA sample analysis has greater clinical potential than FFPE, as results from FNA testing guide surgical decisions. Diagnostic methods range from detection of simple DNA point mutations to comprehensive panel testing which detect a range of DNA, RNA and miRNA changes.

The BRAF V600E mutation occurs in 60-80% of patients with papillary thyroid cancer and is typically present in nodules with Bethesda categories of V (suspicious for malignancy) and VI (malignant). It is rarely present in indeterminate Bethesda categories III-IV. BRAF testing of FNA samples may have clinical utility and guide clinical decision making. Another emerging area for molecular diagnostics in thyroid cancer is in the measurement of miRNAs in the tissue and in the circulation. These small non-coding RNAs are critical for a variety of cellular processes and whilst their exact roles are not well understood they can be detected in the blood and as such have potential for non invasive screening for thyroid cancer presence and or progression.

The development of sensitive molecular diagnostic tools for analysis of FNA samples and the potential utility of serum miRNAs as biomarkers for thyroid cancer presence have the capacity to advance thyroid cancer diagnosis.

**S26 MOLECULAR TESTING IN PHAEOCHROMOCYTOMA**

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Introduction

This year marks the 15th anniversary of a major discovery by Baysal (USA) which changed the genetic landscape of two rare tumour types, inextricably linking them and three other tumour types. Prior to 2000, 10% of phaeochromocytoma (PC), were associated with the familial cancer syndromes MEN2, VHL, or MEN1. Similarly paragangliomas (PGL) of the parasympathetic system were known to occur in families but the genetic basis remained elusive despite extensive linkage studies. Baysal’s discovery in 2000 identified the gene encoding succinate dehydrogenase subunitD (SDHD) as responsible for familial head and neck PGL, and subsequent studies linked these tumours & inherited PC not only to SDHD but also to the other members of mitochondrial complex II, namely SDHC, SDHD and SDHA.

Methods

In 2000 we commenced genetic testing in PC/PGL patients initially for germline mutations in *RET, VHL, SDHD, SDHB, SDHC* and subsequently *SDHA, SDHAF2, TMEM127, MAX* and *EPAS1*. The identification of gastrointestinal stromal tumours, renal cell carcinoma and pituitary tumours with specific pathologies as being SDH deficient, has also expanded the fields of study.

Results

Since 2000 we have tested 604 PC/PGL cases and discovered germline mutation in 163 patients. Together with results from predictive testing in relatives of these probands we have identified 509 individuals with a germline mutation in a PC/PGL gene. To further characterise the phenotype and genotype of these tumours in the Australian population we established the Australian SDH Consortium.

Conclusion

The contribution of genetics to the clinical management of patients and their families with these tumours is a remarkable journey, leading to these tumour syndromes now being recognised as the most highly heritable cancers and exemplifying once again how rare cancers can open a Pandora’s box of discovery.

**SYMPOSIUM 6 – SRAC SYMPOSIUM**

**S27 METANEPHRINE TESTING - WHY, HOW AND WHEN?**

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Various biogenic amines and their metabolites are measured for the biochemical diagnosis, and the monitoring of treatment, of neuroendocrine tumours. The AACB Biogenic Amines Working Party (BAWP) continues to monitor the analytical performance of these tests through the relevant EQA programs offered by the RCPA QAP Chemical Pathology group.

The recent publication by the Endocrine Society of clinical practice guidelines for the investigation of phaeochromocytoma and paraganglioma has confirmed the importance of testing for metanephrines (also called metadrenalines), rather than catecholamines. A metanephrine profile (normetanephrine, metanephrine and 3-methoxytyramine) can be obtained either from a 24-hour urine collection, or from plasma collected under appropriate conditions. At present, urine testing involves an acid hydrolysis step to measure the sum of free and conjugated metanephrines, whereas plasma metanephrines are measured as free compounds.

The BAWP has noted the rise of LC-MSMS as an important analytical technique. The proportion of laboratories enrolled at RCPA QAP in 2015 and using LC-MSMS is 10% for urine free catecholamines, 36% for urine total metanephrines and 85% for plasma free metanephrines. Nevertheless, measurement conditions are not standardised, as illustrated in a survey of laboratories performing plasma metanephrine testing across Australia.

The RCPA QAP EQA program for plasma metanephrines has continued to grow internationally with many overseas enrolments. This reflects both the increased uptake of this test by laboratories and the limited number of suppliers of an EQA program for these analytes.

Future directions predicted for metanephrines, which should assist laboratories, are the use of free, rather than total, urine metanephrine measurements, and the possibility of spot, rather than 24-hour, urine collections. Before this can be achieved, appropriate reference ranges need to be derived from large clinical studies to establish diagnostic test sensitivity and specificity for neuroendocrine tumours.

**S28 QUALITY OF HBA1C MEASUREMENTS IN AUSTRALIA**

A REPORT FROM THE RCPA QAP Working Group

P Williams, S Kerger, V Braniff, on behalf of the RCPA QAP Working Group

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Clin Biochem Rev 36 Suppl 2015 S15
**Introduction**

In 2012 the RCPAQAP introduced a fresh whole blood HbA1c program to supplement the existing program based on lyophilised samples. We report on the findings of this program.

**Methods**

Blood collections were made from volunteer patients with a HbA1c concentration from 8.8 to 11.3%. Aliquots were distributed within 24-48h and results returned in either NGSP% units or IFCC mmol/mol units for statistical analysis and reporting. The data in this abstract is presented in NGSP% units.

**Results**

Participating laboratories increased from 111 in 2012 to 132 in 2015 by which time 52% reported in IFCC units. The total sample CVs (including within- and between-instrument, and between-method variation) ranged between 2.9 and 4.5% with an average of 3.5%. Bias compared with a certified reference method (RefMeth) was: All Laboratory Mean=-0.994xRefMeth+0.06. Performance within manufacturer groups was good with average sample CV and correlations for larger participants as follows: BioRad (Cation Exchange) Ave CV=2.6%, Mean=0.94xRefMeth-0.17 (+0.09 average bias when RefMeth = 6.50%); Roche (Integra): Ave CV=2.7%, mean=0.974 xRefMeth+0.20 (+0.03); Siemens(DCAs): Ave CV=2.8%, mean=0.970xRefMeth+0.172 (+0.00); Allere(afinion): Ave CV=2.7%, mean=0.959xRefMeth+0.20* (-0.07).

The Afinion results in late 2013 showed a low bias of 0.4% units. The company has subsequently corrected this issue (*affected data removed from results above). A sample with haemoglobin F introduced significant bias in some methods reducing accuracy but not affecting precision. With an assigned target value of 8.1%, BioRad Cation Exchange gave 8.3±0.16 and immunoassays gave the following lower results: Siemens DPC (n=26) 7.76±0.16; Roche Cobas (N=16) 7.8±0.3 for this sample.

**Conclusion**

The HbA1c results produced by routine laboratories in Australia are of a high quality. Different technologies can produce acceptable analytical performance when performed correctly although HbF samples can affect some methods. Laboratories should ensure selection of appropriate methods and use to maintain ongoing quality of results.

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**SYMPOSIUM 7 – BONE HEALTH**

**S30 BIOCHEMICAL ASSESSMENT OF “BONE HEALTH”**

**EM Lim**

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Maintaining bone health is a continuum whereby young children should achieve peak bone mass and bone loss in the elderly should be minimised. With our growing ageing population, it is important to identify at-risk individuals of developing osteoporosis and fragility fractures. The assessment of bone health should address risk factors of bone loss by clinical history and examination findings, supplemented by investigations such as bone mineral density (BMD) and biochemical assessment.

Calcium homeostasis and vitamin D status are integral in maintaining bone health. Biochemical markers of bone turnover can complement BMD in identifying patients at risk of fracture and the rate of bone loss, monitoring compliance and response to osteoporosis treatment. We should recognise the effort world experts and organisations trying to standardise bone turnover markers.

In Western Australia, Fasting Metabolic Bone Study is an effective laboratory based interpretative service whereby biochemical markers will be utilised in the investigation and management of patients with metabolic bone disease.

Calcium homeostasis is assessed by serum ionised calcium, plasma total calcium, phosphate, intact parathyroid hormone (PTH), 25-hydroxyvitamin D, and urinary excretion of calcium and phosphate. Our experience with the use of bone turnover markers will be presented.

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**S31 HYPOPHOSPHATAEMIA**

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Recognition and management of hypophosphataemia represents a classic opportunity for partnership between clinicians and clinical biochemists. Too often, either physicians fail to recognize those clinical scenarios for which measurement of serum phosphate is crucial; or biochemists fail to recognize the difference between critical vs incidental hypophosphataemia. Mild hypophosphataemia is often simply due to hyperventilation (and transcellular transfer of phosphate); but low phosphate in a subject presenting with bone pain, myopathy and/or fractures is a cardinal feature of osteomalacia and must be recognized and investigated appropriately by stepwise assessment of urinary phosphate (corrected for GFR), serum 25-hydroxyvitamin D, calcium, PTH and then other more specialized tests (serum FG23, EPG, urine amino acids, and scans such as DOTATAT-PET). Clinical scenarios of hypophosphataemia will be presented to illustrate how appropriate management can dramatically transform clinical outcomes.

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**S32 CALCIUM-SENSING RECEPTORS: MAKING SENSE OF CALCIUM REGULATION**

**AD Conigrave**

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Regulation of extracellular calcium is coordinated by calcium-sensing receptors (CaSRs) expressed in cells of the parathyroid, renal tubules, bone (osteoclasts and osteoblasts) and thyroid (C cells). CaSRs exert physiological actions via tissue-specific signalling pathways. The key effects can be interpreted with reference to the plasma levels of calcium, phosphate and creatinine, serum levels of parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D, urinary calcium excretion, and serum and urine bone turnover markers including ostease and PINP (formation) and CTX and DPD (resorption). In certain clinical contexts, the serum calcitonin level can also be informative. The calcium-sensing receptor coordinates interactions between macronutrient amino acids and calcium metabolism and may be disinhibited in the context of secondary hyperparathyroidism in patients on low protein diets. The parathyroid also accepts inputs from the plasma phosphate level and the phosphate-regulating...
SYMPOSIUM 8 – QA UPDATE

S34 COMMON RESULTS: ARE WE GOOD ENOUGH YET?
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In 2013 the RCPAQAP Chemical Pathology introduced a new program with the aim of providing commutable samples to allow valid comparison of results across different methods. This Liquid Serum Chemistry program offers 2 fresh frozen serum specimens for the analysis of general biochemistry and endocrine analytes. The use of commutable material has been recognised as an important component in External Quality Assurance programs. Associated with this program is a Reference Interval Survey which has provided valuable information about current reference intervals in use in Australia and New Zealand. Last year the RCPAQAP presented at the AACB conference on the common Reference Intervals currently in use and whether these Reference Intervals are introducing additional bias. This year a new addition was made available; the Patient Report Assessment Survey. For this program we asked laboratories to generate a hard copy patient report using the Liquid Serum Chemistry results. We were then able to compare general data displayed on patient reports among laboratories in Australia and New Zealand. This allowed for a review of analyze names in use (e.g. Adjusted Calcium or Corrected Calcium), sequence of analytes on the report (e.g. Alphabetical or Grouped in function), methods of flagging abnormal results (e.g. High/Low, Asterisk and/or Bolding) and units in use (Mass or Molar). This assessment will be helpful in the ongoing work being performed by the RCPA PITUS Working Group on Safety in Pathology Reporting and the AACB’s efforts in harmonising laboratory results.

S35 INTERPRETING EQA AND ASSAYS AT RISK
T Badrick
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Quality Assurance Programs may be directed towards Proficiency testing or External Quality Assurance (EQA) and both can be used to assess risk to patients. EQA programs provide information on the performance of individual laboratories against their peers and ongoing assessments of all methods. QA programs may suffer from specific issues as they are not true patient samples and different programs may be constructed to provide more information about linearity than others. A laboratory can use the EQA data to identify particular assays which are at risk in a structured way and a variation of a risk calculation can be undertaken to stratify assays by risk based on capability. This presentation will describe a visual method of assessing assay performance and a risk calculator which may be used to assist in identifying poorly performing assays.

SYMPOSIUM 9 – VITAMIN D 2015

S36 LC-MS VITAMIN D – A GAME CHANGER FOR LABS
M Wright
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The ever increasing interest in Vitamin D and the measurement of its metabolites over the past decade has made it a “hot topic” in Clinical Chemistry and Endocrinology laboratories around the world. Whilst the large-scale sample volumes lent towards automated chemistry platforms, the requirements for accuracy, reduced imprecision and specificity actually paved the way for LC-MS technology to enter many clinical laboratories for the first time. Following the introduction of the CDC’s Vitamin D Standardization-Certification Program (VDSCP) & a number of Immunoassays successful participation in the scheme, along with changes introduced by the Medical Services Advisory Committee to 25OH Vitamin D requesting, questions have been raised as to the future role LC-MS/MS plays in measuring these analytes. This presentation will cover a brief history of LC-MS/MS for 25OH Vitamin D analysis, the methodology employed at SEALS Prince of Wales Hospital in Sydney, the pros & cons of using this technology and future work in the field.

S37 HUMAN HEALTH AND VITAMIN D STATUS; ‘WHY VITAMIN D TRIALS AND META-ANALYSES OFTEN INDICATE NEGATIVE RESULTS’
HA Morris
SA Pathology and University of South Australia, Adelaide, SA 5000, Australia

The well characterised endocrine pathway of vitamin D metabolism and its activities are responsible for vitamin D regulation of plasma calcium and phosphate homeostasis under control of serum 1,25-dihydroxyvitamin D, the biologically active metabolite of vitamin D. This pathway protects against the metabolic bone disease of osteomalacia in adults or rickets in children. The critical level for serum 25-hydroxyvitamin D to maintain adequate serum 1,25-dihydroxyvitamin D is 20 nmol/L. In contrast a large body of data demonstrate associations between an adequate vitamin D status and incidence of osteoporosis as well as many other diseases. The strongest evidence is for osteoporosis, which extends to the relationship between serum 25-hydroxyvitamin D and bone mineral density and reduction of fracture risk. Bone cells metabolise 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D to elicit biological responses including osteoblast maturation, reducing bone resorption, and enhancing mineral retention in bone to reduce the risk of fracture in the elderly. The critical level for serum 25-hydroxyvitamin D for optimising the health of the skeleton is approximately 60 nmol/L. Vitamin D is a threshold nutrient such that an adequate level is required and excess nutrient is catabolised. In osteoporosis there is a critical interaction between vitamin D metabolism and dietary calcium intake. Without an understanding of the physiological relationship between these nutrients any beneficial outcomes on bone tissue can be obscured. Consequently badly designed randomised clinical trials or meta-analyses of such data will produce an incorrect conclusion. The highest quality clinical investigations must be a test of the underlying physiology in the clinical context. Ignorance of the threshold nature of vitamin D and calcium as nutrients, as well as the critical relationship between these nutrients has been the basis for numerous ‘negative’ outcomes of trials of vitamin D supplements.

S38 VITAMIN D: HYPE, HOPE AND REALITY
CM Florkowski
Canterbury Health Laboratories, Christchurch, New Zealand

Vitamin D has been traditionally linked to bone health, with deficiency causing rickets and osteomalacia. Increasingly, however it has become realised that vitamin D controls the activity of over 200 genes and it has become implicated in a wide range of disease processes, including diabetes, cardio-vascular disease, malignancy and infectious diseases. Coupled with this, is the debate over what constitutes a health plasma 25(OH) vitamin D concentration and whether an optimal level is 50 nmol/L, 70 nmol/L or whatever. If higher concentrations are accepted as healthy, then that renders a significant proportion of our populations as deficient, certainly during winter months and brings into discussion, ways of raising vitamin D, including diet and supplements. These are subject to biases from many confounding factors and reverse-causality, not to mention publication bias. Ideally, a role for vitamin D in any condition should be supported by high quality randomised controlled trials (RCTs). Often, however these are underpowered, of too short duration or use inadequate doses. A paradigm in is infectious diseases where a role for vitamin D has been advocated on the basis of mostly case-control studies and limited RCTs. Our group undertook an RCT of 322 healthy adults, participants, randomly assigned to initial dose of 200 000 IU then 100 000 IU monthly or placebo for 18 months. There were no statistically significant differences in the number of upper respiratory infections, duration of symptoms or days off work between...
the two groups. These findings remained unchanged when the analysis was repeated by season and by baseline 25-OHD levels.

The emergence of high quality RCTs is putting the role of vitamin D into better perspective.
(JAMA 2012;308:1333-9)

SYMPOSIUM 10 – QAP CLINICAL CASES

S39 QAP CLINICAL CASES
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Interpretative commenting is an important aspect of the post-analytical phase in chemical pathology. The introduction of new and complex tests together with increased electronic data communication, use of expert systems, the ability to build interpretative algorithms into laboratory systems and the introduction of clinical and regulatory guidelines are some of the factors that enhance the importance of adding comments to chemical pathology reports. Commenting on reports is a skill that needs to be acquired during training and continually refined. The RCPAQAP Chemical Pathology Patient Report Comments Program is an educational self-assessment program aimed at the continuing professional development of individuals who currently add comments to reports generated by their laboratories, and the education of individuals who are training for such a role.

Several cases which have previously been distributed in this program will be presented in the QAP Clinical Cases Symposium and discussed with active audience participation. Dissenting views will be encouraged and an interactive format will be followed.
PROFERRED ORAL PAPERS

CONCURRENT SESSION 1

A1 DRIED BLOOD SPOT 17 HYDROXYPROGESTEROEONE MEASUREMENT UTILISING ON-LINE SPE COUPLED TO UPLC-MS/MS
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Introduction
Current mass spectrometry methods for Dried Blood Spot (DBS) 17 Hydroxyprogesterone (17OHP) measurement involve labour intensive liquid-liquid extraction (LLE), dry down, reconstitution and long run times. In this study, a method for measuring 17OHP in DBS has been developed that takes advantage of the capabilities of an online SPE system (ACQUITY UPLC Online SPE Manager) to enable the accurate measurement of 17OHP and overcomes the problems associated with LLE.

Methods
DBS samples were mixed with deuterated internal standard in a Zinc Sulfate/Methanol solution in 2 mL 96 well plates. After centrifugation, an aliquot of sample was injected into the online SPE equipped UPLC-MS/MS system. Samples underwent an automated Solid Phase Extraction (SPE) utilising a Waters Online Sample Manager, coupled to a UPLC reverse phase chromatographic system. Quantification of 17OHP was achieved by monitoring two transitions on a Waters Xevo TQD mass spectrometer. The assay run time was 4.5 minutes.

Results
The analytical range of the assay was 500 nmol/L. The Limit of the Blank (LOB) was 0.02 nmol/L and the Limit of Detection (LOQ) with a CV of 20% was 0.20 nmol/L. The inter-run imprecision (CV) for quality control samples at 2.5, 25 and 60 nmol/L was less than 6%. Correlation with the CDC newborn screening quality assurance program based upon 30 samples: UPLC-MS/MS = 0.36 ± 1.03 CDC (r = 0.997).

Conclusion
This assay showed excellent linearity and precision at lower levels and over an extended range. Minimal sample preparation and the utilisation of on-line SPE have produced an efficient and rapid method to measure 17OHP in DBS.

A2 PLASMA FREE METANEPHRINES BY LC-MSMS: COMPARISON OF METHODS IN USE ACROSS AUSTRALIA AND NEW ZEALAND
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Introduction
Recent clinical practice guidelines recommend that plasma free metanephrines should be used for the biochemical diagnosis of pheochromocytoma and paraganglioma. Although nearly all laboratories now utilise LC-MSMS for their measurement, there are many analytical variables to consider when performing this diagnostic test. The AACB Biogenic Amines Working Party decided to survey member laboratories to determine the degree of harmony that exists for analytical factors associated with their performance across Australasia.

Methods
Laboratories were surveyed to obtain detailed information on variables such as how plasma samples were collected and stored, analytes measured and their ion masses, the type of chromatography and mass spectrometer, sources of calibrators and quality controls, and reference ranges used to interpret results. QAP data from Cycle 13 of 2014 were also examined to assess relative performance of the different methods.

Results
Laboratories varied in their compliance to guidelines for specimen collection and processing, such as blood taken supine and stored cold until centrifugation in <6 hours. Both off- and on-line types of SPE were in use for plasma clean-up. There were 2 modes of HPLC: Waters, Thermo and AB Sciex instruments used HILIC chromatography, while Agilent used reverse-phase, which separated metanephrine peaks. Analytical sensitivities were comparable, even with the different tandem mass spectrometers and their patented electrospray ionization source designs. Laboratories found between 7 and 20% of plasma normetanephrine results were high per run while using similar reference ranges. In cycle 13 of the RCPAQAP for plasma metanephrines, most results were within the allowable limits of performance.

Conclusion
Across Australasia, a variety of LC-MSMS instruments and methods are being used to measure plasma free metanephrines, with two distinct modes of HPLC. Despite their differences, QAP data suggested that all laboratories in the survey produced results that were fit for the purpose of pheochromocytoma screening.

A3 EVALUATION OF ABBOTT ARCHITECT URINE FREE CORTISOL CUT-OFF
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Introduction
Urine Free Cortisol (UFC) is one of the screening tests recommended for Cushing’s syndrome. We compared the performance of Abbott Architect unextracted UFC assay with Roche Cobas e411 extracted assay and LCMS assay. The cut-off for Abbott was evaluated.

Methods
Consecutive UFC requests from Royal Melbourne Hospital and Austin Hospital were analysed locally, aliquots were frozen and transported on dry ice for analysis on the other two methods. The cut off for the assays were: LCMS user defined <150 nmol/day, Roche manufacturer recommended <380 nmol/day and Abbott manufacturer recommended <487 nmol/day. Clinical records were reviewed, and samples classified as 1) Cushing or 2) unlikely to be Cushing by two independent endocrinologists. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), ROC was calculated for each method.

Results
Of 69 urine samples from 61 patients, 31 were positive on at least one method, and 12 were true positives. Roche flagged 26 positive results, followed by Grace with 14, followed by Abbott with 12 positive results. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), ROC was calculated for each method.

Conclusion
Abbott manufacturer recommended cut-off (<487 nmol/day) was too high and missed 67% of subjects with Cushing’s. This is undesirable as UFC is used as a screening test to rule out Cushing’s syndrome. A cut-off of <280 nmol/day improved negative predictive value comparable to LCMS.

A4 HORMONE MODELLING IN PRETERM NEONATES: ESTABLISHMENT OF SERUM STEROID REFERENCE INTERVALS BY LC-MS/MS
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Introduction
Current mass spectrometric methods for the measurement of steroid hormones are now widely used in clinical practice. However, recent changes in the screening of neonatal adrenal insufficiency have increased the need for the establishment of reference intervals for serum steroid hormone concentrations in preterm neonates.

Methods
Analytical sensitivities were the same for all methods, but different sample preparation steps were used, including extraction. Both LCMS and LCMS/MS analyses were performed on a Xevo TQD mass spectrometer coupled to an auto-sampler, and results were calculated using Analyst software. Statistical analyses were performed using GraphPad Prism.

Results
The analytical ranges for all analytes were established, and the results were compared with those from previously published studies. The reference intervals for all analytes were found to be within the normal range for term neonates. The inclusion of preterm neonate samples in the reference interval study allowed for the establishment of more accurate reference intervals for these analytes.

Conclusion
The established reference intervals for serum hormone concentrations in preterm neonates will be useful for the diagnosis and management of adrenal insufficiency in this population.
**Introduction**

Immaturity of the endocrine system and its potential impact on morbidity is the subject of numerous studies. Reports suggest significant differences in serum levels of hormones in extremely preterm compared to late preterm and full term infants. The aim of this study was to develop mass spectrometry based reference intervals for five serum steroid hormones from very and extremely preterm infants.

**Method**

Blood was collected from 248 (128 male, 120 female) preterm neonates born between 24 and 32 weeks’ gestation. Participants were recruited from three neonatal intensive care wards in Melbourne Australia. No infant in this cohort had ambiguous genitalia or other endocrine abnormality. All infants included in the reference interval determination survived beyond the equivalent of term. Serum was analysed by LC-MS/MS for analysis of 17 hydroxy-progesterone (17OHP), androstenedione, cortisol, cortisone and testosterone. The method was standardised using the AbsoluteIDQ® Steroid Calibrators, a seven level calibrator set purchased from BIORACRES Life Sciences AG, Innsbruck, Austria). The robust method was applied to define the central 95% reference interval, after each hormone measure was transformed using a Box-Cox transformation to correct for asymmetry.

**Results**

Reference intervals were established for five hormones. Gender specific intervals were developed for testosterone. Cortisone and 17-OHP required division based on gestational age, with neonates born <30 weeks’ gestation demonstrating higher levels than their older counterparts. Androstenedione and cortisol did not require any division within this cohort for reference interval assignment.

**Conclusion**

This report provides the first characterisation of serum steroids measured by mass spectrometry in preterm neonates for infants born ≤32 weeks’ gestation. Utilisation of this data allows for correct interpretation of results for very preterm neonates and reduces the risk of incorrect diagnosis due to misinterpretation of data.

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**CONCURRENT SESSION 2**

**B1 ANTIBODY INTERFERENCE IN ACTH IMMUNOASSAY COMPLICATING MANAGEMENT OF CUSHING’S DISEASE**

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**Introduction**

Antibody interference in immunoassays is a well-known issue in the clinical laboratory, potentially leading to significant clinical impact.

**Case**

A 54-year-old woman with obesity, hypertension, type 2 diabetes mellitus and dyslipidemia was investigated for Cushing’s syndrome. Screening tests revealed elevated midnight salivary cortisol (21 nmol/L; RI <9), 24-hour urine free cortisol (429 nmol/L; RI 25-180), failure to suppress post-1mg dexamethasone (serum cortisol 961 nmol/L; RI <50) and inappropriately elevated ACTH 9.9 pmol/L (RI 1.6-13.9), consistent with ACTH-dependent Cushing’s syndrome. High-dose dexamethasone suppression testing was suggestive of pituitary Cushing’s (>50% decrease in serum cortisol). Although pituitary MRI did not show any lesions, inferior petrosal sinus sampling showed significant central-to-peripheral gradient with lateralisation to the left. The patient underwent a partial hypophysectomy via trans-sphenoidal surgery (TSS). Post-operatively, she was weaned off anti-hypertensive and diabetic medications. Serial serum cortisol levels showed no biochemical cure. She underwent a completion hypophysectomy with a second TSS but post-operative ACTH levels were persistently elevated at 20 pmol/L (on a different platform). An ectopic ACTH source was excluded by negative CT chest/abdomen/pelvis and PET scans so immunoassay interference was investigated. Serial dilution of patient serum showed increasing recovery in ACTH measurements. ACTH measurement on a different assay was much lower (1.7 pmol/L). Incubation of patient serum with heterophile blocking tube resulted in an unexpected increase in ACTH measurement, suggestive of atypical heterophile antibody interference.

**Discussion**

There have been three published cases of ACTH assay interference. Interestingly all involved assays from the same manufacturer. If the endocrinology team had not contacted the laboratory with suspicion of ACTH interference, the patient might have had further unnecessary surgery or investigations with potentially detrimental effects.

**Conclusion**

Clinicians and laboratory staff must communicate rapidly to avoid patient harm when results are discrepant. This case highlights the importance of partnerships in testing between clinical chemistry and endocrinology.

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**B2 CORTICOSTEROID-BINDING GLOBULIN CLEAVAGE IS PARADOXICALLY REDUCED IN ALPHA-1 ANTRTRYPSIN DEFICIENCY: IMPLICATIONS FOR CORTISOL HOMEOSTASIS**

_MA Nenke,1,2 M Holmes,1,2 W Rankin,1,2,3 JG Lewis,2 DJ Torpy,1,2_

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**Introduction**

Alpha-1 antitrypsin deficiency (AATD) is an autosomal codominant condition that predisposes patients to early-onset emphysema and cirrhosis. Alpha-1 antitrypsin (AAT) is the major inhibitor of the destructive serine proteinase neutrophil elastase (NE). Corticosteroid-binding globulin (CBG) regulates the delivery of anti-inflammatory cortisol to tissues and is cleaved by NE at sites of inflammation, resulting in permanent transition to a low cortisol-binding affinity form, and releasing free cortisol. We hypothesised that deficiency of AAT should lead to increased NE activity and therefore increased CBG cleavage in vivo, with decreased absolute and relative levels of the native high-affinity CBG form (haCBG) and increased low-affinity CBG (laCBG), with important implications for the pathogenesis and treatment of AATD.

**Methods**

We performed a prospective observational study of 10 patients with stable AATD and 28 healthy controls. Total CBG, haCBG and laCBG forms were measured in plasma by in-house parallel monoclonal ELISAs.

**Results**

Mean AAT levels were 0.30 g/L; participants with PiZZ mutations had significantly lower AAT levels than SZ or MZ mutations. Mean ± SEM circulating levels of total CBG were similar among AATD patients and controls (512 ± 46 and 498 ± 15 nmol/L; P=0.8), but haCBG was significantly higher (353 ± 36 and 264 ± 8 nmol/L; P<0.005), and laCBG lower (159 ± 19 and 225 ± 11 nmol/L; P=0.016) in the AATD group. The ratio of haCBG:totalCBG was significantly higher in AATD (69 ± 3% and 54 ± 1.3%; P=0.0001). There was a negative correlation between the ratio of haCBG:total CBG and AAT levels (P<0.05, R=0.67).

**Conclusion**

Despite a lack of AAT and excess uninhibited NE, CBG cleavage is paradoxically reduced in AATD under basal conditions with increased absolute and relative levels of haCBG compared with controls. The pathogenic implications for cortisol delivery under conditions of acute or subacute infection require further study.

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**B3 LABORATORY MANAGEMENT OF HAEMOLYSED SAMPLES – RESULTS OF AN RCPAQAP SURVEY**

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**Introduction**

Incident data submitted through the RCPAQAP Key Incident Monitoring and Management Systems (KIMMS) program over the last four years has consistently highlighted haemolysis as a major source of test/specimen rejection. KIMMS data has consistently shown sample rejection rates of 17% (8% due to pre-analytical errors). KIMMS data indicates rejection. KIMMS data has consistently shown sample rejection rates of 17% (8% due to pre-analytical errors). KIMMS data indicates rejection. KIMMS data has consistently shown sample rejection rates of 17% (8% due to pre-analytical errors). KIMMS data indicates rejection. KIMMS data has consistently shown sample rejection rates of 17% (8% due to pre-analytical errors).
as much as 30-40% of all rejected samples are haemolysed.

Methods
A haemolysis survey was electronically distributed to all laboratories enrolled in each discipline of the RCPAQP to gauge the impact of haemolysis across all areas of pathology. The survey was conducted in 2010 and a less detailed version was conducted in 2014 to compare results.

Results
Survey results showed an increase in the number of laboratories utilising the Haemolysis Index of instrumentation to determine haemolysis rather than relying on visual inspection of the sample. Results also show that there is a wide variation in the cut-off value used for determining haemolysis in a sample. As many as 60% of laboratories do not report haemolysis rates outside the laboratory despite most haemolysed samples originating from areas outside the control of the laboratory. While the survey indicated a wide range of haemolysis rejection policies within laboratories, there are still approximately 10% of laboratories that do not have a haemolysis rejection policy.

Conclusion
This survey demonstrated the variation in reporting of results on haemolysed samples and the apparent lack of policies on haemolysed samples.

B4 SUPPORTING GLUCOSE TESTING OVER 1 MILLION SQUARE KILOMETRES

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Introduction
Monitoring of blood glucose levels is important for managing and maintaining normalised blood glucose concentrations in hospitalised patients. For the majority of sites in Country Health South Australia (CHSA), glucose testing relies solely on Point of Care equipment. To ensure glucose results are managed safely and effectively, we have implemented a robust management program.

Methods
After evaluation of current glucose methods, the Nova StatStrip was chosen as the connectable meter of choice for CHSA. Meters were implemented across all hospitals and connected to downloaders for network access, allowing integration to the existing Integrated Point of Care Clinical System (iPOCCS). An electronic Basal Bolus Insulin (BMI) dosing calculator was incorporated into the iPOCCS system utilising Nova glucose results. Quality management was applied through internal quality control (QC) review incorporated in to the iPOCCS system with automatic downloading and external quality assurance (EQA) program participation including bi-monthly reports.

Results
110 Nova glucose meters have been implemented across CHSA. Internal QC samples are tested at two levels each day, with results automatically downloaded in to iPOCCS for analysis through statistical and graphical review. Alerts are sent for any sites missing their required QC result and for any out of range results. All patient glucose results automatically downloaded in to an electronic patient record on the iPOCCS system for review by nursing, general practice or specialist staff. For patient’s requiring BMI dosing, glucose results are automatically printed on to the electronically generated chart, listing recommended insulin dosing. The EQA program consists of two samples every two months with comprehensive reports generated and sent out to sites in the month following testing.

Conclusion
The use of network connectivity, database integration and strong quality control and assurance policies have enabled the effective management of glucose testing for CHSA patients across one million square kilometres.

B5 ANALYSIS OF TROTONIN T DELTA CHANGES FOR THE DIAGNOSIS OF AMI

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Introduction
A rise and/or fall of troponin with at least one value above the 99th percentile limit is essential for the diagnosis of Acute Myocardial Infarction (AMI) in patients presenting with symptoms suggestive of Acute Coronary Syndrome (ACS) when using high sensitive assays. However, there are no clear criteria for what constitutes a significant delta change and decision limits in the literature are varied.

Methods
Blood samples drawn at 7 time points post admission were collected from 806 patients. Plasma samples were tested on the Roche eletcys 2010 high sensitive troponin T laboratory assay. Data was evaluated using absolute delta change (5, 7 and 10 ng/L) and relative delta change (10%, 20% and 50%) as recommended in the literature and ROC analysis was used to determine the optimal delta change at each time point.

Results
Results showed thirty-nine patients (4.8%) diagnosed with AMI. 7 ng/L obtained the best results for absolute delta change and 20% produced the best results for relative delta change. Absolute delta change produced significantly better results compared to relative change. At the 4 hour time point, ROC analysis produced area under the curve of 0.949 for 7 ng/L absolute delta change compared to 0.782 for 20% relative delta change. Sensitivity (94.6% vs 67.6%), specificity (95.3% vs 88.7%), positive predictive value (41.7% vs 25.3%) and negative predictive value (99.7% vs 98.0%) were all better for absolute change compared to relative delta change. The optimal absolute delta change for each time point increased as the timing to the second sample became longer.

Conclusion
In this study, absolute delta change was clearly superior when compared to relative delta change for the diagnosis of AMI. Delta changes should be used in an appropriate way as part of a clinical pathway for the diagnosis of AMI and prevent unnecessary hospital admissions.

C1 MODIFIED ABBOTT C16000 URINE PROTEIN METHOD FOR PATIENTS WITH DILUTE URINE

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Introduction
Analysis of urine protein/creatinine ratios is an increasingly used parameter in lieu of 24hr urine protein collection, particularly in the diagnosis of pregnancy associated proteinuria. In patients with a combination of low urine protein concentration and dilute urine (i.e. low creatinine concentration), the final calculated ratio may need to be reported as “less than” a value which is higher than the clinical cutoff (e.g. <35 mg/mmol, when values >30 mg/mmol suggest increased pregnancy risk). This situation can be particularly confusing for clinicians.

We noted during a change of method from Roche Integra to Abbott Architect a loss of sensitivity at low concentrations that would increase the likelihood of this scenario (Roche 0.04 g/L, Abbott 0.068 g/L). In our patient population as many as 40% of our urine protein requests have a concentration reported (from Roche Integra) of <0.07 g/L.

Method
In response, Abbott Australia developed a modified urine protein method with increased (3x) sample/reagent ratio, specifically for dilute urine estimations. In this scenario (Roche 0.04 g/L, Abbott 0.068 g/L). In our patient population as many as 40% of our urine protein requests have a concentration reported (from Roche Integra) of <0.07 g/L.

Results
Analysis of urine protein/creatinine ratios with an increasing parameter in lieu of 24hr urine protein collection, particularly in the diagnosis of pregnancy associated proteinuria. In patients with a combination of low urine protein concentration and dilute urine (i.e. low creatinine concentration), the final calculated ratio may need to be reported as “less than” a value which is higher than the clinical cutoff (e.g. <35 mg/mmol, when values >30 mg/mmol suggest increased pregnancy risk). This situation can be particularly confusing for clinicians.

We noted during a change of method from Roche Integra to Abbott Architect a loss of sensitivity at low concentrations that would increase the likelihood of this scenario (Roche 0.04 g/L, Abbott 0.068 g/L). In our patient population as many as 40% of our urine protein requests have a concentration reported (from Roche Integra) of <0.07 g/L.

Method
In response, Abbott Australia developed a modified urine protein method with increased (3x) sample/reagent ratio, specifically for dilute urine estimations. Only samples with values measured <0.068 g/L were to be re-tested by the modified methodology.

Results
Linear regression by Passing-Bablok between Architect (original method) and Integra across a range of concentrations (0.04 to 10.0 g/L) showed excellent agreement (Slope 1.157, intercept – 0.030, n=30). Within run precision (n=20) value on 2 dilute patient samples were as follows:
**Table 1**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Precision @ 0.04 g/L</th>
<th>Precision @ 0.08 g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integra</td>
<td>23.6%</td>
<td>7.6%</td>
</tr>
<tr>
<td>Architect (original)</td>
<td>(below reporting limit)</td>
<td>6.8%</td>
</tr>
<tr>
<td>Architect (modified)</td>
<td>3.2%</td>
<td>(not run)</td>
</tr>
</tbody>
</table>

**Conclusion**

Some manufacturers’ urine proteins assays do not have suitable characteristics to confidently identify at risk patient groups, particularly pregnant women, in dilute samples. We found a modified assay could deliver significantly improved low concentration sensitivity and imprecision. Ideally all assays should offer sufficient low end sensitivity without compromising high end linearity to avoid the need for a second estimation via a modified methodology.

**C2 MEASUREMENT UNCERTAINTY FOR THE CLINICAL LABORATORY – A REVISION OF THE CONCEPT**

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**Background**

The aim of estimating the uncertainty of a measurement (MU) is to assist with interpreting results. In the metrological concept uncertainty is considered relative to the ‘true value’ of the measurement, value which cannot be known but is that which would be obtained by a perfect method (without bias or imprecision). I propose that the concept of the ‘true value’ in laboratory medicine should be modified in different settings.

**Methods**

The different types of result comparisons used for interpretation of numerical laboratory results are: 1. Comparison with a previous result on the same patient; 2. Comparison with a population reference interval; 3. Comparison with a clinical decision point. A different concept of true value is proposed for each setting.

**Results**

1. The true value is defined by the method used to produce the previous result. For patient at a single laboratory the true value is the performance of the analyser itself and consideration of bias is inappropriate. 2. The true value is defined by the method used to produce the reference interval. As reference intervals should be validated for use in a laboratory the true value can again be considered to be the laboratory method. Inclusion of the uncertainty of transferring a reference interval may be appropriate. 3. The true value is defined by the method used in the clinical studies used to derive the decision point. This is different to the setting of reference intervals as it is not possible to establish or confirm clinical decision points in routine laboratories.

**Conclusion**

The determination of MU in the clinical laboratory should include consideration of the use of the results. As different comparators are used for different decisions, variations in the concept of MU may provide the most useful information. Traceability of all methods would remove this added complexity.

**C3 THE RELATIONSHIP BETWEEN ROUTINE VITAMIN B6 LEVELS AND NON-P5P TRANSAMINASE ASSAY ACTIVITY**

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**Introduction**

Medicare Australia has recently approved rebates for holotranscobalamin (HoloTC) or methylmalonic acid (MMA) as confirmatory tests when serum total B12 (TB12) is low or equivocal. We examined (1) How well do different TB12 assays agree? (2) Is HoloTC better than TB12 for diagnosis of B12 deficiency? (3) What are the equivocal TB12 ranges for the commonly used assays?

**Methods**

Serum samples (n=195) selected from routine TB12 requests were sent frozen to different laboratories for the analysis of HoloTC (Abbott Architect), MMA (GCMS) and TB12 using Roche (e602), Abbott (Architect), Siemens (Centaur) and Beckman (DXI800). B12 deficiency was defined as MMA>0.5 μmol/L. ROC curves were used to indicate B12 sufficiency or deficiency and the limits of the equivocal range.

**Results**

(1) Abbott TB12 results were similar to Roche (Abbott=1.02Roche:+2.6 pmol/L). Siemens TB12 were similar at values >200 pmol/L (Siemens=0.85Roche:+50 pmol/L) but appeared to be proportionally higher at values ≤200 pmol/L. Beckman-TB12 results were approximately 35% lower (Beckman=0.76Roche-20 pmol/L). (2) The area under the ROC curve was significantly higher for HoloTC (0.87) than for all of the TB12 assays (Roche: 0.79; Architect: 0.81; Centaur: 0.75; Beckman: 0.79). (3) The equivocal ranges from ROC curve analysis for TB12 (pmol/L) were: Roche 115-272, Abbott 121-268, Siemens 137-263 and Beckman 64-176. The equivocal range for HoloTC was 22-53 pmol/L, rounding to 25-50 pmol/L.

**Conclusion**

HoloTC has better diagnostic accuracy for vitamin B12 deficiency than current TB12 assays when increased plasma MMA is used as the reference point. Significant differences exist between different TB12 assays meaning that equivocal ranges are assay-dependent.
ASSESSING THE QUALITY OF LABORATORY MEASUREMENTS OF HORMONES IN THE 2015 RCPA QAP ENDOCRINE PROGRAM

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Introduction

The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) conducts External Quality Assurance (EQA) annually for the measurement of endocrine hormones in human serum. For the 2015 cycles, high-accuracy Reference Measurement Procedures (RMPs) developed by the National Measurement Institute (NMI) were used to assign reference values for cortisol, testosterone, 25-hydroxyvitamin D₃ (25OHD₃) and 17β-estradiol in four of six lyophilised samples distributed to participants. The results of laboratories were evaluated against the reference values.

Methods

Reference values were determined by isotope dilution-liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis employing single and highly selective dual column chromatography systems. Certified Reference Materials (CRMs) for testosterone, cortisol and 25OHD₃ in human serum were analysed to verify the trueness of the RMPs. The measured analyte mass fractions in the CRMs were within 98-101% of the certified values demonstrating minimal bias in the RMPs.

Results

Laboratories participating in the EQA predominantly used electrochemiluminescence and luminescent immunoassay platforms for analysis. A few laboratories (<10 of up to ~180 participants) used LC-MS/MS. The medians of laboratories’ results showed positive deviations from the reference values for cortisol (3-17%) and testosterone (1-22%) over the range of concentrations analysed. For 25OHD₃, negative deviations (10-30%) that increased with increasing concentration were observed. For 17β-estradiol, laboratory medians were 20-93% higher than the reference values. The coefficients of variation in participants’ results also ranged from 8-47% for the four analytes.

Conclusion

The EQA highlighted differences between the reference values assigned to samples and the results of laboratories. These differences may be due to cross-reactivity issues and the incomplete extraction of analytes from binding proteins that can compromise assay performance. The differences may be also attributable to possible non-commutability of lyophilised samples. A commutability study is required to determine whether the observed differences are related to the EQA samples or are genuine biases in laboratory methods.
P1 URINARY CATECHOLAMINES MEASUREMENT BY LC/MS/MS

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Introduction

Analysis of urinary catecholamines (i.e. epinephrine, norepinephrine and dopamine) is used for the diagnosis pheochromocytoma and paraganglioma which are catecholamine-producing tumours. Although liquid chromatography with electrochemical detection (HPLC-ECD) represents one of the most commonly used techniques for measuring urinary catecholamines, it is laborious, time-consuming and technically demanding. Our aim was to develop and validate LC/MS/MS method for the quantification of free catecholamines in urine in order to replace the HPLC method.

Methods

Five hundred microlitre of 24 hour urine sample was pre-purified by Bond Elute Plexa SPE cartridge, using phenylboronic acid complexation. Reversed phase (pentfluorophenylpropy1 column) chromatography was applied. Mass spectrometric detection was operated in multiple reactions monitoring mode using a quadrupole tandem mass spectrometer with positive electrospray ionization. Results obtained by LC/MS/MS method were compared with liquid chromatography with electrochemical detection.

Results

Total run-time was 6.5 min. Intra- and inter-assay analytical variation was less than 5%. Linearity ranged from 5ug/L to 1000ug/L with R² values of 0.99 or greater. Relative recovery of the biogenic amines by the SPE column ranged from 103% to 110%. Matrix effect was studied and ranged from 5% to a maximum of -20%. Method comparison was studied by using 50 samples and 50 RCPA samples. Bland-Altman plot and Deming regression analysis was performed. LC/MS/MS method correlated well with HPLC-ECD method.

Conclusion

The advantages of LC/MS/MS method for catecholamine include its high analytical performance by selective phenylboronic acid affinity and high specificity and sensitivity by unique MS/MS fragmentation.

P2 URINARY METANEPHRINE BY LC/MS/MS FOR CLINICAL USE

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Introduction

In June 2014, the Endocrine Society published its first clinical practice guideline for pheochromocytoma and paraganglioma (PPGL). In the guideline it has been mentioned that the initial biochemical testing for PPGLs should include measurements of plasma free or urinary fractionated metanephrine. They recommended the use of liquid chromatography with mass spectrometric (LC/MS/MS) or electrochemical detection (HPLC-ECD) method for the measurement of plasma or urine metanephrines. The aim of this work was to implement simple LC/MS/MS method for measuring metanephrines in urine as a routine service.

Methods

Urine samples were extracted using Bond Elute Plexa SPE cartridge, using phenylboronic acid complexation. Urinary fractionated metanephrines were quantified using Agilent 6460A Triple Quad LC/MS/MS System (California, USA) coupled with Agilent 1290 UHPLC system.

Results

All compounds eluted within 3.5 minutes. Recoveries ranged from 96-110%. Absolute ion suppression and matrix effect were observed, however, were completely compensated for by the internal standards. Matrix effects for metanephrine, normetanephrine and 3-methoxy tyramine were -14%, -16% and -21% respectively. Calibration curves were linear from to 10 to 1000 µg/L for all analytes with R² values of 0.99 or greater. Precision of the method was checked as per EP 05-A2 protocol and the CV was less than 5%. 50 patient urine samples and 50 RCPA EQA samples previously analysed by HPLC-ECD, were reanalysed by the LC/MS/MS method. Bland-Altman analysis showed bias for metanephrine, normetanephrine and 3-methoxytyramine were -6.2%, 2.2% and -3.8% respectively. Deming regression analysis showed these two methods correlated well.

Conclusion

The LC/MS/MS method is precise, linear and with short duration of analysis. Hence this method is suitable for routine clinical use.

P3 NEW CUT-OFF VALUE OF THE VIDAS CMV IgG AVIDITY GIVE LESS BORDERLINE RESULTS

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Introduction

CMV IgG Avidity is still a major assay to aid in differentiation between primary and non-primary CMV infection. Cut-off value being used in a particular system is crucial in determining the result interpretation which eventually will be influential towards patient management, particularly for pregnant woman. Recently, Vidas CMV IgG Avidity assay has applied new cut-off with narrower borderline range (0.40 ≤ index < 0.65) compared to the previous cut-off (0.20 ≤ index ≤ 0.80). The new cut-off is determined based on the study of CMV IgG kinetics. This study was conducted to evaluate the performance of the new cut-off Vidas CMV IgG avidity assay in comparison with previous Vidas cut-off and other system (Architect, Abbott).

Methods

CMV IgG Avidity from human sera (n=40) was measured using Vidas and interpreted by both the new and old Vidas cut-off. Most samples with discordance results are also conducted by Architect system.

Results

The agreement between the new and old Vidas cut-off is 77.50% (31/40). Nine discordances are caused by borderline result in old cut-off become low avidity (1 sample) and high avidity (8 samples) in new cut-off. The number of borderline result in new cut-off decline by four-fold compared to the old one. Meanwhile, the agreement between Architect and Vidas (both new and old cut-off) remains similar (42.11%). The low agreement is caused by many low avidity results in Architect becoming borderline or high avidity in Vidas.

Conclusion

The new cut-off of Vidas gives the less borderline results than the old cut-off. This less number of borderline results are expected to gain better patient care management. Compared with Vidas, Architect has a tendency to giving more low avidity results, but this requires further study to investigate.

P4 MULTISTAGE FRAGMENTATION OF 1α, 25(OH)2 VITAMIN D3 COMBINED WITH METABOLITE PROFILE BY LCMSMS UTILISING A QUADRUPOLE LINEAR ION TRAP

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Introduction

1α, 25(OH)2 vitamin D3 is the most biologically active form of vitamin D. However, low levels of observed ionization results in low intrinsic response for this metabolite when measuring by tandem mass spectrometry (LC-M/SMS). This, together with very low plasma concentrations, potential of interfering peaks and background noise, makes it a challenging analyte to investigate by LC-M/SMS. Enhancement of ionisation can be achieved by the introduction of derivatisation steps or by encouraging in-source adduct formation, however, issues with selectivity remain. In this study we investigated the use of multistage fragmentation (MRM) with a quadrupole linear ion trap (QLIT) instrument to enhance analyte selectivity.
Methods
Vitamin D metabolites were obtained from patient plasma by liquid/liquid extraction prior to chromatographic separation on a PFP column and analysed on a Scion QTRAP 6500. Quantification of the 1α, 25(OH)2-vitamin D3 was conducted by both MRM m/z transitions m/z 399 > 135 and MRM(3) transitions m/z 399 > 135 > 117.

Results
Under the conditions described an LOQ of approximately 30 pmol/L for 1α, 25(OH)2-vitamin D3 is readily achievable with no discernable matrix interference. The enhanced selectivity of the multistage fragmentation also gives rise to the added benefit of differentiating from isobaric dihydroxy metabolites such as the 24, 25(OH)2-vitamin D3. Additionally, the separation by the PFP column prior to quantification allows not only the evaluation of multiple vitamin D metabolites but also the epimeric forms that have been highlighted as important in paediatric evaluation.

Conclusion
The use of the MRM(3) technology allows the analysis of non-derivatised 1α, 25(OH)2-vitamin D3, without adduct formation. This powerful new tool also improves the analytical selectivity whilst retaining the required analytical sensitivity.

P5 STEROID ANALYSIS IN DIFFERENT MATRICES UTILISING STANDARDISED ON-LINE SPE COUPLED TO UPLC-MS/MS
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Introduction
Steroid analysis in plasma, urine, saliva and ultra-filtrate utilise a number of different extraction procedures that are time consuming and inefficient. The ability to extract different matrices under uniform conditions and also run them under the same chromatographic conditions is a major advantage. The analysis of cortisol, cortisone and prednisolone using this standardised approach will be discussed. In this study, a method for measuring these analytes in different matrices has been developed that takes advantage of the capabilities of an online SPE system (ACQUITY UPLC Online SPE Manager) to enable their accurate measurement.

Methods
Urine, plasma, saliva and ultra-filtrate samples were mixed with deuterated internal standard in a Zinc Sulfate/Methanol solution in 2 mL 96 well plates. After centrifugation, an aliquot of sample was injected into the online SPE equipped UPLC-MS/MS system. Samples underwent an automated Solid Phase Extraction (SPE) utilising a Waters Online Sample Manager, coupled to a UPLC reverse phase chromatographic system. Quantification of cortisol, cortisone and prednisolone was achieved by monitoring two transitions on a Waters Xevo TQD mass spectrometer. The assay run time was 4.5 minutes between injections.

Results
The analytical range of the assay was 5000 nmol/L. The Limit of the Blank (LOB) was 0.05 nmol/L and the Limit of Detection (LOD) was determined as 0.25 nmol/L. The inter-run imprecision for matrix matched quality control samples were all less than 8 %.

Conclusion
This assay showed excellent linearity and precision at lower levels and over an extended range. Minimal sample preparation and the utilisation of on-line SPE have produced an efficient and rapid method to measure Cortisol, Cortisone and Prednisolone in Urine, Plasma, saliva and ultra-filtrate. The concept of a standardised approach for sample preparation has been extended to 17 hydroxyprogesterone, androstenedione, testosterone, DHEAS, DHEA, DHT and progesterone being assayed within the same run.
Conclusion

Whilst we will extend our study to look at potentially problematic samples such as with increased WCC, this study raises the possibility of a shorter spin time to decrease the TAT for urgent specimens.

P8 ROUTINE LIPOID PARAMETERS IN THE PREDICTION OF SMALL DENSE LDL

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Introduction

Modifications of LDL increase atherosclerotic risk apparently because they increase uptake of LDL cholesterol through macrophage scavenger receptors. Genetic modifications include ApoB mutations or Lp(a), while the most common acquired modification seems to be small dense LDL (sdLDL), which is associated with the dyslipidaemia in insulin resistance. While fasting serum triglyceride levels are predictive of the presence of sdLDL, we sought to evaluate the other routine lipid parameters as predictors of the amount of sdLDL.

Method

Routine lipids, which included total cholesterol (TC), triglycerides and HDL cholesterol (HDLc), were analysed on Roche c701. Calculated routine parameters include LDLc (Friedewald), LDLc/HDLc ratio, TC/HDLc ratio and non-HDLc. sdLDL was measured on the same samples using the Lipoprint® gradient gel electrophoresis. Spearman rank correlations were performed.

Results

295 samples were assessed (105 women and 190 men). All the routine parameters were correlated with high significance (p<0.001) to the level of sdLDL, in increasing order of prediction: HDLC (-0.33), TC (0.35), LDLc (0.41), triglycerides (0.45), non-HDLc (0.48), LDLc/HDLc (0.56) and TC/HDLC (0.58).

Conclusion

While simple measures such as TC, HDLC and triglycerides correlate with the amount of sdLDL, calculated parameters seem better. Non-HDLc seems superior to triglycerides, but not as good as LDLc/HDLc and TC/HDLc seems the best predictor.

P9 EVALUATION OF THE PRECISION AND ACCURACY OF HbA1C ASSAYS

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Introduction

With HbA1c now used for diagnosis in addition to monitoring of diabetes mellitus, assay precision and accuracy are important, as small variations in measurement can have a significant impact on patient classification. We evaluated new and current HbA1c assays.

Methods

New HbA1c assays including Abbott Architect (enzymatic), Bio-Rad D100 (HPLC cation exchange), Sebia Capillaries 2 FLEX-PIERCING (capillary electrophoresis) and Trinity Premier H9210 (boronate affinity) were compared to the existing HbA1c methods including Roche Integra800 (turbidimetric inhibition immun assay), Arkray Adams HA-8160 (HPLC) and BioRad Variant II Turbo 2.0 (HPLC). Fresh whole blood samples with HbA1c ranging from 29-87 mmol/mol (4.8-10.1%) were collected from staff and BioRad Variant II Turbo 2.0 (HPLC). Fresh whole blood samples with HbA1c ranging from 29-87 mmol/mol (4.8-10.1%) were collected from staff.

Over the range of HbA1c tested, inter-assay CV [median (range)%, in NGSP units] in ascending order were: Architect 0.9 (0.6-1.2); D100 0.9 (0.8-1.3); Arkray 1.1 (0.8-1.5); Capillaries 1.2 (0.5-1.2); Integra800 1.2 (0.9-2.2); Premier 1.7 (1.6-3.0) and VariantII 1.7 (1.4-2.5). Against the IFCC targets, the equations (Passing&Bablok) were: Architect=1.06-0.46x; D100=1.00x-0.02; Arkray=1.01-2.9x; Capillaries=1.05-0.30x; Integra800=0.98+0.07x; Premier=1.07-0.36x, and VariantII=0.98+0.04x. Applying these equations, at the diagnostic cut-off of 6.5%, six of the seven methods achieved absolute bias of ≤±0.2%. Similar accuracies were obtained against the RCPA-QAP WB3-04, with a target of 6.8%. The RCPA-QAP lyophilised glycohaemoglobin materials gave greater bias for all methods.

Conclusion

The Architect, D100, Arkray, Capillaries and Integra showed superior precision compared to our current method (VariantII). Almost all assays had bias ≤±0.2% at the diagnostic cut-off. Fresh whole blood material is better than the lyophilised glycohaemoglobin material for accuracy assessment.

P10 PARAPROTEIN INTERFERENCE IN AUTOMATED CHEMISTRY

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Introduction

Paraproteins are known interferents in immunoassays and chemistry assays mainly due to immune complex formation and precipitation. They may also cause spectral interference in automated analysers that use photometric measurements. We report a case of a patient whose serum GGT and urea were repeatedly undetectable due to undiscovered IgM myeloma. Methods

The spectral absorbance curves for serum GGT and urea assays (Siemens Advia) for a 79 year old male patient whose GGT and urea values were undetectable were compared with absorbance curves with patients with high and normal GGT and urea levels. A high total protein and globulin level in the index patient serum lead to serum electrophoresis which uncovered an IgM myeloma. Absorbance curves for serum samples with normal total protein and globulin were examined after spiking with the patient’s serum or IgM quality control material.

Results

Serum electrophoresis using alkaline buffered agarose gel uncovered IgM κ and λ, with a combined concentration of 28g/L. The absorbance curve for the patient’s serum for both GGT (at 478nm) and urea (at 410nm) assays were markedly different to those of serum with normal protein and globulin levels. Spiking normal serum with the patient’s serum or IgM quality control material did not cause a significant change in GGT or urea, other than a dilution effect.

Conclusion

The absorbance curves for the patient provides convincing evidence of a spectral interference caused by the paraprotein. To our knowledge this is the first report of Siemens Advia GGT and urea being affected by IgM paraproteins. Fictitious results are likely to cause unwarranted interventions, and could cause harm. Astute and observant scientists who identify these before reports are released will avoid unnecessary interventions and anxiety.

P11 RESPINNING LITHIUM HEPARIN TUBES AFTER REFRIGERATION FOR ADD ON REQUESTS

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Introduction

Lithium Heparin (Becton-Dickinson PST) tubes are our preferred sample type due to the prevalence of anticoagulant therapy within our hospital. Once refrigerated these sample present a problem with add-on requests due to the precipitation of fibrinogen in the separated plasma. To avoid this problem we aliquot and centrifuge the samples prior to analysis. In a busy automated laboratory this involves a number of bottlenecks that consume time and staff. It also involves the introduction of potential error in mislabeling and incorrect aliquoting. In this study we investigated the effect of respinning the primary tubes to resolve these issues and improve laboratory process.
**Methods**

Patient samples, prior to discard (approximately 7 days refrigeration), were aliquoted and then both the primary tubes and aliquot tubes were centrifuged. After centrifugation, samples were visually inspected for appropriate settling and then assayed for twenty five analytes (representing a range of molecular forms and properties). A second respin of the primary tube was also performed to measure the effect of multiple add-ons over a period of time.

**Results**

LDH and Potassium demonstrated occasional unacceptable imprecision between the aliquoted sample and the respun primary tubes. However, all other analytes demonstrated acceptable precision at spin times of 10, 5 and three minutes (2400g).

**Conclusion**

For all analytes investigated, other than LDH and Potassium, respinning the primary tube was an acceptable and convenient process. To avoid erroneous results for add-on LDH and Potassium, respun tubes should be clearly marked and a recollect requested if no other sample is available. Analysis of one year’s add-on requests showed that this would result in the refusal of approximately 20 add-on requests for LDH per annum.

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**Methods**

Cardiac troponin tests may be performed on different analysers within the same health service. In this setting it is important to be aware of how results from different methods compare. Additionally, knowledge of sample type acceptability and stability can allow release of more results. We analyse these factors for the Abbott i-STAT analyser troponin I, especially by comparison with Roche troponin T.

**Methods**

Samples measured on the i-STAT for troponin I were also measured on the Roche high-sensitivity assay on the E170 analyser (hs-TnT) and the Cobas h232 cartridge based system. Hs-TnT was used as the reference standard. Serum was assessed for suitability and stability for the i-STAT assay.

**Results**

A total of 84 samples were measured on the i-STAT of which 83 were also measured for hsTnI and 28 on the h232. Using a reference interval of 0.04 µg/L (i-STAT) and 14 µg/L (hsTnI), TnI had 72% sensitivity and 100% specificity. The similar values for the h232 using 50 µg/L (hs-TnI) and 14 µg/L (hs-TnT), TnI had 72% sensitivity and 100% specificity. The similar values for the h232 using 50 µg/L (hs-TnI) and 14 µg/L (hs-TnT), TnI had 72% sensitivity and 100% specificity. The similar values for the h232 using 50 µg/L (hs-TnI) and 14 µg/L (hs-TnT), TnI had 72% sensitivity and 100% specificity.

**Conclusion**

The i-STAT TnI method using a decision point of 0.04 µg/L is more sensitive than using the decision point of 0.08 µg/L while maintaining 100% specificity. The i-STAT TnI method using a decision point of 0.04 µg/L is more sensitive than using the decision point of 0.08 µg/L while maintaining 100% specificity. The i-STAT TnI method using a decision point of 0.04 µg/L is more sensitive than using the decision point of 0.08 µg/L while maintaining 100% specificity.

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**Methods**

Five leftover urine samples with varying concentrations of creatinine, sodium, potassium, chloride, calcium and phosphate were enriched with boric acid to final concentrations of 10g/L and 20g/L, representative of 1L and 0.5L of 24-hour urine collection respectively. All analytes were measured on the Beckman Coulter AU5810 analyser. The recoveries of the six analytes were expressed as percentages of the native samples.

**Results**

The average recoveries of the boric acid enriched urine samples are as follows: creatinine (98%), sodium (100%), potassium (98%), chloride (100%), calcium (99%) and phosphate (97%).

**Conclusion**

This study showed that the addition of high concentrations of boric acid did not impact on the measurement of urinary calcium, chloride, phosphate, creatinine and sodium.

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**Methods**

The evaluation protocol included precision studies using cosabs b 101 controls (L1 and L2 Lot # 004108-01) and patient comparison studies using Bio Rad D10 ion exchange chromatography and Siemens DCA Vantage immunoassay.

**Methods**

The precision data is as follows: a) consecutive run (n=9) L1 5.4±0.13, CV 2.4%; L2 9.8±0.21, CV 2.1%, and b) between day (n=10) L1 5.2±0.12, CV 2.3%; L2 9.7±0.15; CV 1.5%. Comparison studies using patient EDTA whole blood samples: a) versus (x) Bio Rad D10 (n=44) (y-cobas b 101) yielded the following Passing-Bablok equation y=0.903x+0.49 (95% CI for intercept 0.145-0.838 and slope 0.862-0.947) with an analytical range of 4.3-12.4% Hb, bias -0.3±0.5% Hb; b) versus (x) Siemens DCA Vantage (n=38) (y-cobas b 101) yielded the following equation y=1.000x+0.100 (95% CI for intercept -0.213-0.642 and slope 0.923-1.059) with an analytical range of 4.3-11.8% Hb, bias 0.1±0.5% Hb.

**Conclusion**

The study data indicates there is strong agreement between the Roche cosabs b 101 HbA1c with both the Bio Rad D10 and the Siemens DCA Vantage methods, especially in the range between 6-10% by visual inspection. The analyser is very easy to use with the only precaution being that the disc must be inserted within 60 seconds after sample application.

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**Methods**

For all analytes investigated, other than LDH and Potassium, respinning the primary tube was an acceptable and convenient process. To avoid erroneous results for add-on LDH and Potassium, respun tubes should be clearly marked and a recollect requested if no other sample is available. Analysis of one year’s add-on requests showed that this would result in the refusal of approximately 20 add-on requests for LDH per annum.

---

**Methods**

Cardiac troponin tests may be performed on different analysers within the same health service. In this setting it is important to be aware of how results from different methods compare. Additionally, knowledge of sample type acceptability and stability can allow release of more results. We analyse these factors for the Abbott i-STAT analyser troponin I, especially by comparison with Roche troponin T.

**Methods**

Samples measured on the i-STAT for troponin I were also measured on the Roche high-sensitivity assay on the E170 analyser (hs-TnT) and the Cobas h232 cartridge based system. Hs-TnT was used as the reference standard. Serum was assessed for suitability and stability for the i-STAT assay.

**Results**

A total of 84 samples were measured on the i-STAT of which 83 were also measured for hsTnI and 28 on the h232. Using a reference interval of 0.04 µg/L (i-STAT) and 14 µg/L (hsTnI), TnI had 72% sensitivity and 100% specificity. The similar values for the h232 using 50 µg/L (h232) were 45% and 100%. If the i-STAT was used at the manufacturer’s 99th centile of 0.08 µg/L the sensitivity fell to 41%. Serum gave equivalent results to heparinised whole blood and was stable for up to 7 days at 4 degrees.

**Conclusion**

The i-STAT TnI method using a decision point of 0.04 µg/L is more sensitive than using the decision point of 0.08 µg/L while maintaining 100% specificity. It is also more sensitive than the h232 TnT assay. Serum was shown to be a suitable alternative to heparinised whole blood for TnI testing on the i-STAT. The stability of TnI in serum allows add-on tests for routinely stored samples.

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**Methods**

Cardiac troponin tests may be performed on different analysers within the same health service. In this setting it is important to be aware of how results from different methods compare. Additionally, knowledge of sample type acceptability and stability can allow release of more results. We analyse these factors for the Abbott i-STAT analyser troponin I, especially by comparison with Roche troponin T.

**Methods**

Samples measured on the i-STAT for troponin I were also measured on the Roche high-sensitivity assay on the E170 analyser (hs-TnT) and the Cobas h232 cartridge based system. Hs-TnT was used as the reference standard. Serum was assessed for suitability and stability for the i-STAT assay.

**Results**

A total of 84 samples were measured on the i-STAT of which 83 were also measured for hsTnI and 28 on the h232. Using a reference interval of 0.04 µg/L (i-STAT) and 14 µg/L (hsTnI), TnI had 72% sensitivity and 100% specificity. The similar values for the h232 using 50 µg/L (h232) were 45% and 100%. If the i-STAT was used at the manufacturer’s 99th centile of 0.08 µg/L the sensitivity fell to 41%. Serum gave equivalent results to heparinised whole blood and was stable for up to 7 days at 4 degrees.

**Conclusion**

The i-STAT TnI method using a decision point of 0.04 µg/L is more sensitive than using the decision point of 0.08 µg/L while maintaining 100% specificity. It is also more sensitive than the h232 TnT assay. Serum was shown to be a suitable alternative to heparinised whole blood for TnI testing on the i-STAT. The stability of TnI in serum allows add-on tests for routinely stored samples.
and the acceptability criteria was 90% concordance for qualitative results and Pearson correlation co-efficient R ≥0.9 for quantitative results. 10 urine samples were run on both Cobas 6500 and Iris and correlated with manual microscopy performed by a single operator to confirm correct identification. Between-day precision over 10 days was determined using Biorad Liquicheck urinalysis controls for urine red blood cells (uRBC), urine white blood cells (uWBC), glucose, protein, bilirubin, urobilinogen, pH, specific gravity (SG), ketones, nitrates and leukocytes. Statistical analysis was performed using Microsoft Excel.

Results
There was good correlation between Cobas u701 and iQ200 Elite for uRBC (γ=0.906x – 1.634, R=0.89) and uWBC (γ=0.989x + 0.521, R=0.97). Cobas u601 correlated well with iChem Velocity for pH (γ=0.983x + 0.087, R=0.986), SG (γ = 0.992x + 0.0099, R = 0.969), glucose, protein, bilirubin, urobilinogen, ketones, nitrates and leukocytes (≥90% concordance). Urine microscopy results correlation between the automated instruments and manual microscopy was good with ≥90% concordance. Between-day precision was good with ≥90% concordance for qualitative results and <15% for uRBC and uWBC at concentrations of 201 and 23 per high power field respectively.

Conclusion
The analytical performance met our expectations for a fully automated urine analyzer and we hope to determine its operational characteristics such as turnaround time in future.

P16 CASE REPORT: MACRO-TROPONIN T

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Introduction
Macro-complexes (immunoglobulin-protein complexes) are a recognised cause of interference with laboratory results. We describe a case of persistently elevated troponin T (TnT) which were out of keeping with the clinical picture, with subsequent demonstration of a macro-TnT complex.

Methods
A 76 year old man was admitted to hospital following an episode of chest pain on a background of multiple cardiovascular risk factors. The TnT concentration on admission was 907 ng/L falling to 753 ng/L over the next 5 days (Roche high-sensitivity TnT assay). Laboratory suspicion was raised when negative results for TnT were obtained using the Roche h232 analyser. Further testing to determine the cause of the discordant results was undertaken, together with a review of the medical records.

Results
The elevated TnT measurements were linear on dilution. Testing of samples for Troponin I on four different analytical systems all produced results within the respective reference intervals. Sample pre-treatment with specific heterophile antibody blocking tubes (Scantibodies®) did not diminish analyte recovery. PEG precipitation demonstrated decreased recovery (≤20%) in contrast to normal patient controls (57–143%). On review of the medical records, the patient previously had 20 persistently elevated troponin T results as part of 6 presentations over the course of 4 years, with all levels between 440–950 ng/L.

Conclusion
The findings of persistent elevation of hsTnT, normal results for Troponin I, linear dilution, lack of effect from blocking reagent and precipitation with PEG are all consistent with macro-TnT. The negative results with the h232 analyser may be due to the macro-TnT failing to migrate in the lateral flow cartridge. This is the first described case of a macro-TnT to our knowledge. The clinical pathway of this case is similar to that seen with previously described macro-

P17 CSF BILIRUBIN MEASUREMENTS ON ROCHE MODULAR – EFFECT OF ASSAY REFORMULATION

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Introduction
Automated measurement of CSF bilirubin on the Roche Modular using a modified application of the serum bilirubin assay has been used to rule out CSF xanthochromia without scanning spectrophotometry. In 2014, Roche updated the formulation of the assay (Bilirubin Total Gen.3). The reformulated assay was compared to scanning spectrophotometry for the measurement of CSF bilirubin.

Methods
Over an 8-month period, patient CSF samples collected from the emergency department were analysed in duplicate using the reformulated assay on the Roche Modular. Net bilirubin absorbance (NBA) was also determined on these samples by scanning spectrophotometry. Diluted serum quality control material (Bio-Rad Liquichek Unassayed Chemistry Control (Human) Level 1) was used for quality control of NBA measurement.

Results
Accuracy of bilirubin measurement by spectrophotometry and the Roche method were confirmed by internal QC and EQA. 42 patient specimens had sufficient volume for analysis by both automated bilirubin measurement and scanning spectrophotometry. Considerable scatter was observed in the relationship with the Roche measurements generally higher. Using NBA of 0.007 as the reference standard, a bilirubin concentration decision point of 0.15 µmol/L showed 100% sensitivity and 72% specificity. Decision points of 0.20 µmol/L and 0.25 µmol/L were associated with 85% and 97% specificity respectively with 100% sensitivity for both. Due to low sample numbers, calculated specificities should be considered as estimates.

Conclusion
The Roche Bilirubin Total Gen.3 assay may be used to rule-out CSF xanthochromia using the previous bilirubin concentration cut-off of 0.15 µmol/L. Raising the decision point will decrease the number of false positives whilst maintaining clinical sensitivity. Until more data is available around the 0.007 NBA decision point, bilirubin ≤ 0.20 µmol/L is a reasonable conservative limit for ruling out CSF xanthochromia without scanning spectrophotometry. In comparison to NBA, the higher bilirubin concentrations obtained using the automated assay may be due to analytical non-specificity.

P18 VALIDATION OF BIOCHEMICAL TESTING OF FLUID SAMPLES BY DILUTION INTO A SERUM MATRIX: A PRELIMINARY INVESTIGATION

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Introduction
It is the responsibility of individual laboratories to validate biochemical testing in fluids not specified in the product’s package insert. How best to perform the validation is an area of active interest. In this preliminary study we describe the use of serial dilutions of non-serum fluids into the validated serum matrix to evaluate the suitability of neat fluid analysis using assays intended for serum.

Methods
21 non-serum fluid samples were serially diluted into pooled serum and measured for 25 common analytes on the Roche Modular analyser. Osmolality and pH were also measured in the neat samples. Linear regression of the neat fluid. Assessment criteria were based on RCPAQAP allowable limits of performance (ALP) for serum.

Results
Multiple sample types were analysed, including peritoneal, pleural, drain, and unspecified fluids, with measured osmolality 258–358 mmol/kg and pH 7–10. Direct neat fluid measurements were within ±1 ALP of predicted values for most analytes (Na, K, Cl, bicarbonate, creatinine, urate, glucose, phosphate, Mg, Ca, protein, bilirubin, ALT, AST, alkaline phosphatase, GGT, LDH, CK, lipase, amylase, lactate, triglycerides). Neat measurements of albumin and cholesterol differed from predicted values by >1× ALP in only 1 sample (21 g/L vs. 24 g/L and 1.1 vs. 1.5 mmol/L respectively), with both assays demonstrating good linearity on serial dilution (Pearson correlation coefficient r = 0.994). Urea measurements differed from predicted values by >3× ALP in 2 of 21 samples.
Conclusion
Based on this preliminary investigation, many routine analytes appear suitable for neat analysis in non-serum fluids using these Roche assays intended for serum. Further investigation of the urea assay is required to determine suitability of analysis in non-serum fluids.

P19 PERFORMANCE EVALUATION OF C-REACTIVE PROTEIN AND PROCALCITONIN TEST ON THE ichroma™ Smart ANALYZER
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Introduction
For monitoring infection and inflammation episodes, host response biomarkers such as C-reactive protein (CRP) and procalcitonin (PCT) are now being recognized as useful tools in the diagnostic process. This study was aimed to evaluate the analytical performance of the recently developed, semi-automated ichroma™ Smart system (Boditech Med Inc., Chuncheon-si, Gangwon-do, Korea), which allows interconvertible measurements of CRP and PCT.

Methods
We evaluated the analytical performance and agreement with the laboratory standards of CRP and PCT measurements by the ichroma™ Smart system. Precision and linearity study as well as method comparison with the DxC 800 (Beckman Coulter, Fullerton, CA, USA) for CRP, and with the VIDAS (bioMerieux SA, Marcy-l’Étoile, France) for PCT were performed according to corresponding CLSI guidelines. Additionally, we evaluated the carryover rates between specimens.

Results
Total precision (% CV) in measuring low, middle, and high level controls (level 1, 2, 3) was 6.32%, 5.75%, and 3.56% for CRP, and 8.07%, 6.24%, and 6.53% for PCT using the ichroma™ Smart system. In the linearity test, R² was 0.9995 and 0.9982 when measuring CRP (0.1-225.2 mg/L) and PCT (0.05-60.91 ng/mL), respectively. Good correlation was observed between the ichroma™ Smart and the DxC 800 (r = 0.997) for CRP, and between the ichroma™ Smart and the VIDAS (r = 0.992) for PCT. Carry-over effect was 0.02% for CRP and 0.04% for PCT, respectively.

Conclusion
ichroma™ Smart system for CRP and PCT showed an adequate performance and appeared to be a suitable clinical analyzer with simple operating procedure.

P20 A NOVEL APPLICATION OF LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY TECHNOLOGY FOR ESTABLISHING THE PRESENCE OF TOPICAL STEROIDS IN A SKIN WHITENING CREAM
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Introduction
With liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology evolving into a routine diagnostic tool, the ability of laboratories to identify the presence of biologically active molecules in body fluids and exogenous matrices is greatly enhanced. The flexibility of this powerful analytical tool was demonstrated when a patient presented to Emergency with hyperglycaemia, dehydration, and a cushingoid appearance. As a recent immigrant to Australia, it was established that the patient was regularly applying a product known as ‘skin whitening’ cream. A literature search was conducted to establish ionisation characteristics for high potency steroids. Detection of the steroid was achieved by direct injection of the reconstituted extract and monitoring of ion transitions using the ABSciex-QTRAP-5500. The QTRAP function of Enhanced Product Ion (EPI) scanning was used to acquire full scan MS/MS spectra of the steroid.

Results
Three extractions of the cream demonstrated a significant peak at 5.8 minutes with mass transitions of 467/355 and 467/373. These corresponded to the expected mass transitions for Clobetasol Propionate, a high potency topical steroid. The spectrum in the serum was compared with that seen in the cream confirming the presence of Clobetasol.

Conclusion
Although quantitation was not performed with the method described here, identification of a causative agent provided valuable clinical information and reinforced that the patient should discontinue the use of this non-prescription product. The increased access to ‘non-prescription’ medications via the internet would suggest the incidence of this scenario is likely to increase. This process details how the laboratory can provide rapid and relevant information for clinical decision-making.

P21 N-ACETYLCESTYINE INTERERENCE IN THE ABBOTT AND CAMBRIDGE LIFE SCIENCES PARACETAMOL ASSAYS
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Introduction
The measurement of paracetamol is important for the diagnosis and management of paracetamol overdose, particularly in deciding whether to administer or continue N-acetylcyisteine (NAC) treatment. Due to concerns about negative interference by NAC to the Abbott paracetamol assay, the Cambridge Life Sciences (CLS) paracetamol assay was evaluated. NAC interference studies were also performed.

Methods
The CLS assay was evaluated on the Abbott Architect c16000. Patient sample comparisons were performed against the Abbott and Roche Cobas paracetamol assays (N=20). For the NAC interference studies, aliquots of paracetamol-free serum were spiked with a range of concentrations of paracetamol 110-980 μmol/L and NAC 200-1000 mg/L, and paracetamol measured immediately.

Results
Passing-Bablok equations: CLS paracetamol= 25 + 1.02x Abbott and 27 + 1.05x Roche (μmol/L units). At NAC concentrations of 200, 400, 600, 800 and 1000 mg/L, median recovery values for the range of paracetamol concentrations were 78% (Abbott) vs 97% (CLS), 55% (Abbott) vs 92% (CLS), 44% (Abbott) vs 89% (CLS), 34% (Abbott) vs 85% (CLS), and 27% (Abbott) vs 81% (CLS), respectively.

Conclusion
The CLS paracetamol assay had a constant positive bias compared to the Abbott and Roche assays associated with higher blank measurements in paracetamol-free sera. The CLS assay demonstrated significantly less negative interference with NAC than the Abbott assay and is therefore appropriate in a clinical setting where paracetamol quantitations are required urgently and a proportion of samples are collected from patients who have received NAC treatment.

P22 LOW URINE pH CAN CAUSE FALSE-HIGH MEASUREMENT OF 5-HIAA BY LC-MSMS
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Introduction
When investigating neuroendocrine tumours, common laboratory practice is to require a 24-hour urine collection in a container containing acid. This procedure produces a urine pH between 1 and 3 to stabilise catecholamines from oxidation. The same procedure is used to collect urine for the tumour-marker metabolites vanillylmandelic acid (VMA), homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5-HIAA), which can be assays
simultaneously by LC-MSMS after dilution of the urine. This study has examined the stability of diluted urine samples used for estimation of these metabolites.

**Methods**

Urines were diluted 1:7 with a mixture of 3 deuterated [D] internal standards in water prior to LC-MSMS. VMA, 5-HIAA and HVA were separated on an Atlantis T3 column (50 x 2.1 mm) under isocratic conditions with 20% methanol in 0.2% formic acid, and quantitated by peak area ratios. Diluted urines were stored at room temperature and assayed daily for up to 4 days.

**Results**

Stability studies of 20 diluted urines showed that the concentrations of VMA and HVA did not change significantly over time, but 5-HIAA increased to reach an average of 180% (range 109-388%) of the initial concentration after 2 days. Further investigations revealed that the increase was pH-dependent. The time taken to exceed the initial concentration by >10% was 2 h at pH ≤1.5, 7 h at pH 1.6-2, 17 h at pH 2.1-2.5 and 25 h at pH 2.6-3. The increase was explained after mass spectral studies showed that [D5]-5-HIAA underwent gradual conversion to [D3]-5-HIAA, due to acid-catalysed deuterium-proton exchange with loss of internal standard signal.

**Conclusion**

The accurate measurement of urinary 5-HIAA by LC-MSMS, using [D5]-5-HIAA as internal standard, requires immediate analysis or pH control in order to avoid acid-catalysed deuterium-proton exchange and false-high concentrations. Alternatively, a [13C3]-5-HIAA internal standard should be used.

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**P23 EVALUATION OF THE EFFECT OF ABNORMAL HAEMATOCRIT LEVELS UPON THE ACCURACY OF HOSPITAL GRADE BLOOD GLUCOSE METERS**

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**Introduction**

Some blood glucose meters can give falsely high or low blood glucose readings when a patient has a normally low or high haematocrit levels, respectively. Other interferences from Ascorbic Acid and non-glucose sugars can also lead to inaccurate Glucose readings from some meters. Accurate glucose measurement for acutely ill patients is crucial to their overall care, and hospital grade blood glucose meters have been developed to help with this.

**Methods**

Using the NCCLS protocols, Hospital grade meters from two companies were tested using fresh venous blood samples with low, normal and high haematocrits and blood glucose levels ranging 2.5 mmol/L - 12 mmol/L. Ascorbic acid interference was also investigated.

**Results**

Multi-well technology within the StatStrip test strips measures and corrects for interferences from haematocrit and ascorbic acid leading to accurate blood glucose results that correlate well with the laboratory reference method.

**Conclusion**

The Nova StatStrip Xpress and StatStrip Connectivity meters demonstrated good overall performance with no clinically significant interference due to Haematocrit or other factors. The StatStrip Connectivity version of meter offers additional benefits in terms of positive patient ID and electronic download of results.
P26 REVERSE PHASE COLUMN CHROMATOGRAPHY (RPC) FOR HIGH SPEED SEPARATION OF AMINO ACIDS AND ITS DERIVATIVES WITHIN BASIC AND LIMITED RESOURCE REDUCED SETTINGS

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Introduction

Amino acid analysis is commonly used in proteomics for suspected testing for inborn errors of metabolism, as a powerful technique to discover all 24 amino acids in the samples. A short turnaround time is now feasible for analysis with low sample volume. RPC employ a highly sensitive method, maximising lab productivity. Even in newly established, restricted and limited resources, improved runtime, column longevity and ruggedness of the previous methods employed so far could be focussed on.

Methods

A new methodology using recently developed columns with an engineered particle size of 1.8 micron particles was developed on 1220 Agilent System using only a binary pump and variable wavelength detector using UV:338 nm 10 nm bandwidth (for OPA amino acids):262 nm Reference: 324 nm (for FMOC amino acids). The columns used were Eclipse AAA; 3x150x3.5 microns which can withstand a high pressure of 400bars. Mobile Phase A: 10mM NaHPO4, 10 Mm Na2B4O7, pH 8.2. Mobile Phase B: Acetonitrile methanol: water (45:45; 10 v/v) was made by extra pure reagents & filtered through the 0.22 micron filter.

Results

A 30 minute runtime was given for each calibrator, control and sample. All 24 amino acids were calibrated and the graph obtained. The peaks of all amino acids were appreciated. As a pilot run, 19 samples (15 plasma and 04 urine samples of newborns and infants) were run, with the AUC, the actual concentration of amino acids present.

Conclusion

This rapid and robust way of measurement of the amount of amino acids in plasma and urine samples utilizing minimal resources with customised timetable worksheet helped to attain good calibration curve providing the best assessment tool. Although the convention detector used is diode array detector or fluorescent detector we could accomplish the calibration of all 24 amino acids and sample analysis was satisfactory.

P27 WHICH SPECIMEN TUBE IS BEST FOR SERUM/ PLASMA OR WHOLE BLOOD TRACE ELEMENT ANALYSIS?

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Introduction

Trace elements are typically found in the body in concentrations of <100mg/L and contamination from collection techniques, laboratory environment and plastic/glassware are known complications of trace element analyses. Accurate measurement of these low concentrations necessitates a metal-free testing environment and consumables along with highly sensitive analytical techniques. The aim of this study was to examine the suitability of different collection tubes for trace element analysis in plasma and whole blood.

Methods

Blood from 16 subjects was collected into eight different Greiner Bio-One Vacutte collection tubes (8mL Serum Clot Activator with gel, 8mL Lithium Heparin with and without gel, 8mL K2-EDTA, 5mL K2-EDTA with gel separator, 6mL Sodium Heparin Trace Element, 500µL K2_EDTA Minicollet) and a 500µL BD Microtainer K2-EDTA Low Lead Microtainer. Elements tested were serum or plasma aluminium, chromium, cobalt, copper, selenium and zinc and whole blood cadmium, lead and mercury. Analyses were performed on a Perkin Elmer ELAN DRC ICP-MS using a OneFAST sample introduction system and median values compared between the tube types.

Results

No significant differences were seen across the collection tubes for zinc, copper, cobalt, lead and cadmium. However, median chromium values were significantly higher in serum, p<0.0001. EDTA plasma with gel separator had higher mean values for selenium and cobalt. Aluminium comparisons showed contamination was present across most collection tubes: the better performers being an in-house nitric acid washed plain container and the trace element tube. The Minicollect EDTA samples showed a higher median mercury value.

Conclusion

This evaluation will change the sample collection tubes our laboratory recommends/accepts for trace element analyses. EDTA plasma will not be accepted for aluminium analysis. Serum will not be accepted for aluminium, chromium or cobalt analyses. For adult lead and mercury analyses we will recommend the trace element tube and for paediatric populations the BD Microtainer.

P28 USUAL PATTERN OF SERUM FREE THYROXINE DISTRIBUTION IN CHILDREN ON THYROXINE FOR HYPOTHYROIDISM

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Introduction

We aim to study the typical FT4 and FT3 distribution in children on thyroxine for primary hypothyroidism to guide optimal monitoring for all children on thyroxine, including those with central hypothyroidism.

Methods

FT4 and FT3 results (Beckman Coulter DxI 800 method) over a nine-year period from patients aged 30 days to 19 years with TSH in the normal range (0.4-4.0 mU/L) were extracted from the Monash Pathology database. Patients with primary hypothyroidism were identified by treating physicians or from medical records (thyroxine-treated group). Patients with single thyroid function test, not known to have thyroxine treatment were analysed separately (control group). FT4 and FT3 ranges (median (2.5th - 97.5th)) were calculated. Results of the thyroxine-treated group were compared to the manufacturer’s quoted reference intervals (FT4: 7.9-14.4 pmol/L; FT3: 3.8-6.0 pmol/L) and to the control group.

Results

In the control group, FT4 (n=3128) was 11.1 pmol/L (7.9-16.4) and FT3 (n=660) was 5.6 pmol/L (3.7-7.3). In the thyroxine-treated group (congenital hypothyroidism (n=89) and autoimmune hypothyroidism (n=33)), FT4 (n=747) was 14.5 pmol/L (9.2-22.6) and FT3 (n=146) was 5.6 pmol/L (4.3-7.6) respectively.

Conclusion

In thyroxine-treated hypothyroidism with normal TSH, FT4 levels were 38% higher than the control group and 57% higher than the manufacturer’s reference interval. The corresponding FT3 levels were no different compared to the control group but were 27% higher than the manufacturer’s reference interval. This study suggests that target FT4 in children on thyroxine should be well above the Beckman manufacturer’s quoted reference interval and our in-house control interval. In addition, FT3 intervals for thyroxine-treated children and those not on thyroxine should be higher than the manufacturer’s reference interval.

P29 METROLOGICAL TRACEABILITY OF ARCHITECT ALKALINE PHOSPHATASE AND AMYLASE ASSAYS TO IFCC REFERENCE METHODS

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Introduction

ISO 18153, Metrological traceability of values for catalytic concentration of
enzymes assigned to calibrators and control materials, describes traceability for enzyme assays. The IFCC established enzyme reference measurement procedures (RMPs). ARCHITECT systems (<4000, c8000, c16000) must produce accurate and comparable test results as determined by comparison to the ‘gold standard’ RMPs. Metrological traceability of optimized enzyme calibration factors for alkaline phosphatase (ALP) and amylase (AMY) was established, ensuring agreement with the RMPs.

Methods
Human serum samples were assigned ALP and AMY enzyme activity target values using the primary RMPs (IFCC reference laboratory, Hannover, Germany). Sample aliquots were stored at -75 C before being tested using the field assays on two c8000 systems using different reagent lots in a routine clinical laboratory (Stuttgart).

Results
The mean results from both ARCHITECT systems were compared to the results from the ALP and AMY RMPs. The mean % bias was 1% (95% limits of agreement; range -8.8 to +10.8%) for ALP and 0.1% (95% limits of agreement; range -8.5 to +8.2%) for AMY. Passing-Bablok regression equations: mean ARCHITECT result (y) = -1.09 + 1.02 RMP result (x), n = 57, for ALP, and (y) = 2.35 + 0.99 RMP results (x), n = 51 for AMY. Least squares results for ALP were y = -0.49 + 1.01 x for AMY. The enzyme calibration factors for both were optimized for agreement with the RMPs.

Conclusion
Metrological traceability of ALP and AMY to the internationally recognized RMPs listed in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database was established. The optimized enzyme calibration factors produce results in excellent agreement with IIFCC reference methods. Traceability of enzyme assays is necessary for global standardisation of clinical laboratory practice and direct comparability of patient test results.

P30 ASSESSING ANALYTICAL QUALITY OF Hb A1c ASSAYS USING REFERENCE SAMPLES, ACCURACY BASED GRADING, AND SIGMA METRICS
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Introduction
Hb A1c assays require minimal bias and high precision to diagnose Type 2 Diabetes. Four common Hb A1c assays were evaluated using accuracy based grading and Six Sigma metrics.

Methods
Eight frozen whole blood samples from the European Reference Laboratory (ERL) for Glycohemoglobin with target values assigned by the IFCC Hb A1c reference method were tested. Assays evaluated: Abbott ARCHITECT Next Generation enzymatic; Roche Tina-quant A1c-2; Tosoh G8 HPLC; and Bio-Rad Variant II Turbo HPLC. Reference samples were tested twice in five replicates (n = 10) by each assay. Mean values and % CV were calculated for each sample and assay, and Sigma metrics estimated [Sigma = (TEa – bias)/%CV]. Bias was absolute difference between the target values and observed values, TEa (total error allowable) was 6%.

Results
The observed biases ranged from 0.3 – 2.9% (Architect), 0.8 – 6.8% (Roche), 1.6 – 6.5% (Tosoh), and 0.5 – 3.8% (Bio-Rad). The number of samples for which Sigma metrics were ≥ 6.8/ for Architect (range 3.5 – 30), 2/8 for Roche (range 0 – 7.2), 0/8 for Tosoh (range 0 – 4.2), and 5/8 for Bio-Rad (range 0.4 – 21).

Conclusion
Hb A1c is a critical assay because of the world-wide diabetes epidemic and analytical quality is imperative. The IFCC reference method for Hb A1c is internationally accepted and commutable whole blood reference samples with reference method target values are available. The “true bias” of assays using accuracy based grading, instead of “relative bias” by peer group grading, can be measured. Total analytical quality depends on both bias and precision and Sigma metrics combine both to objectively assess quality. Although an assay may demonstrate acceptable bias, accuracy may be compromised by poor precision. Using accuracy based grading, analytical performance of common Hb A1c field methods compared to the IFCC reference method demonstrated some marked differences.
DBP) but for Total Cholesterol, LDL and FPG showed statistically significant (p<0.05) difference between the two groups. Fifty five percent (n=95) of obese and 2% (n=1) of non-obese children had 2 or more abnormal metabolic parameters (p<0.000) while 8.8% (n=15) of obese and none of the non-obese children had MetS (p=0.015). Of the sub sample of obese children (n=109), 24 (23.3%) had elevated ALT and 26 (25.7%) had elevated AST. Elevated M.Alb/Cr ratio was seen only in one child. Half of the sample had elevated hs-CRP (n=56, 53.8%).

Conclusion

Metabolic abnormalities are present significantly in obese children compared to their non-obese counterparts from a younger age. Liver transaminases and hs-CRP were elevated in a significant number of obese children.

P33 FLAT ORAL GLUCOSE TOLERANCE TEST REVISITED

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Introduction

Gestational diabetes mellitus (GDM) that is defined as glucose intolerance of variable severity with onset or first recognition during pregnancy has a strong association with adverse pregnancy outcomes. The Oral Glucose Tolerance Test (OGTT) is considered as the current ‘gold standard’ for the diagnosis of GDM.

A flat plasma glucose concentration curve is one of the major problems encountered while interpreting OGTT results. It is reported when the glucose concentration at 1 hour is less than 1mmol/L above the fasting value. In an attempt to interpret the flat OGTT, we decided to implement testing plasma insulin for all flat responses.

Methods

Between January and June 2015, 75g 2 hour OGTTs were performed on 1786 pregnant women aged 16-46 years. 206 flat responses were identified.

Results

Based on the insulin response, the OGTT have been divided into 4 subgroups. In Group A (n=111), OGTT showed early insulin release consistent with normal glucose metabolism. Group B (n=57) showed a similar insulin release but further rise of insulin at 2 hours. In comparison, in Group C (n=30), there was relatively less initial insulin release at 1 hour but a further release at 2 hours, consistent with delayed glucose absorption. In the small Group D (n=8) both insulin and glucose concentrations were low, suggestive of poor glucose absorption.

Conclusion

Reflex insulin testing allowed us to identify that 54% of the women did not require a repeat OGTT. However, groups B and C showed evidence of delayed absorption and group D with poor absorption required retesting.

P34 BIO-RAD D100 HBA1C – THE SAME CHROMATOGRAPHY IN HALF THE TIME

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Introduction

The approval of HbA1c for the diagnosis of diabetes has seen a dramatic increase in the number of requests for this analyte. HPLC remains the preferred analysis method for HbA1c due to its ability to detect haemoglobin variants but the prolonged analysis times compared to automated immunoassay methods are a disadvantage in the high throughput laboratory. The Bio-Rad D100 may provide a solution – the manufacturer has optimised the chromatography to significantly reduce the analysis time to 45 seconds per sample but preserving the benefits afforded by chromatography. We undertook a field study to compare the performance of the Bio-Rad D100 against our established method, the Bio-Rad Variant II TURBO.

Method

All routine requests for HbA1c were analysed in parallel over a 2 week period (n=1052). In addition two samples – one with a high and one with a low HbA1c value were tested repeatedly over multiple days to obtain an estimate of imprecision. Additional samples with known haemoglobin variants were also analysed in parallel.

Results

There was good correlation between results from the two instruments, R² = 0.99. The Deming regression equation was D100 (mmol/mol) = 0.900 x Variant II (mmol/mol) + 4.34. The coefficient of variation was 2.8% at 27.7 mmol/mol and 0.9% at 107 mmol/mol. Both instruments flagged the presence of known haemoglobin variants similarly and appropriately. Over the study period, four results were flagged by the D100 where no abnormality was detected by the Variant II.

Conclusion

The new Bio-Rad D100 instrument offers a viable alternative to immunoassay for high throughput measurement of HbA1c. It provides rapid sample processing without significant loss of sensitivity for the detection of haemoglobin variants. The results correlated well with our existing methodology and met current criteria for imprecision.

P35 METFORMIN DELAYS PROGRESSION TO TYPE 2 DIABETES BY 60% – RESULTS OF TEN YEARS OF ROUTINE CLINICAL USE

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Introduction

A large number of type 2 diabetic (T2DM) patients continue not to be diagnosed until they suffer a cardiovascular (CVD) event. Patients with impaired glucose tolerance (IGT), despite having a CVD risk approaching those with T2DM, are currently under-recognised and lack organised follow-up. However, Lipid clinics see many of these patients because of the accompanying dyslipidaemia and following publication of the DPP and UKPDS papers suggesting that progression to diabetes could be delayed and that metformin may reduce CVD in T2DM, a more proactive approach has been taken to treating patients with IGT.

Methods

All patients referred to the Lipid clinics at West Suffolk or Peterborough Hospitals with fasting triglycerides of 3 mmol/L or above were given a 75g oral glucose tolerance test and those diagnosed with IGT were offered extra diet and lifestyle advice and metformin 500 mg bd, if desired. Statistical analysis was by Chi-square test with Yates’ correction for small numbers.

Results

Seventy-one patients have been diagnosed with IGT since 2005 and 52 elected to take metformin (10 later discontinued treatment). The rate of progression to T2DM in the 52 patients was significantly reduced by almost 60% (p <0.003) compared to the diet and lifestyle group – which has previously been shown to develop T2DM at the same rate as expected for an IGT cohort. The reduced rate of progression to T2DM in the metformin group became significantly different after 4 years of follow-up (p <0.025). As in the DPP follow-up, the probability of progression to T2DM was significantly lower (p <0.004) in patients who managed to maintain an FBG <5.6 mmol/L.

Conclusion

This open label study confirms that routine use of metformin significantly delays the progression from IGT to T2DM potentially delaying the development of the long-term consequences of hyperglycaemia.

P36 A PATIENT WITH COMPLETELY UNDETECTABLE HEMOGLOBIN A₁C REPORTED AS ZERO PERCENT

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Case Description

A blood sample from a 2 yr old male patient who presented with fever since 3 days and pallor was assayed for haemoglobin (Hb) A₁c on a Bio-Rad D10 HPLC system. No peak was seen on the chromatogram and the HbA₁c was reported as 0%. A complete blood count revealed Hb concentration to be 6.2 g/dl.
Discussion

The sample was analyzed for hemoglobin variants in the extended mode which eluted with 95.2% HbF and 4.1% Hb A2 and 0.3% HbA. The likely diagnosis was beta thalassemia major. Because there is very less (0.3%) HbA, ion exchange HPLC will not detect glycated Hb in such individuals. There are several other factors that affect HbA results such as homozgyosity for HbE, carboxamylated Hb, any condition that decreases RBC survival.

P37 HBAIC ACROSS FOUR PLATFORMS, THE ABBOTT ARCHITECT ENZYMIC METHOD, BIO-RAD VARIANT HPLC WITH THE ALERE AFFINION AND ROCHE B101 POC IMMUNOASSAY METHODS

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Introduction

HbA1c is the preferred test for the diagnosis and monitoring of diabetes mellitus. There is expected to be an increasing demand on laboratories to perform the test as a routine automated test and in addition there is great interest in Point of Care (POC) testing. In this study we have compared the performance of the new enzymatic HbA1cassay from Abbott with two POC methods (Alere Affinion and Roche b101) compared against our NGSP certified Bio-Rad Variant cation-exchange HPLC method.

Methods

Fifty patient samples (HbA1c range 20-140 mmol/mol), were selected from our routine patient population and analysed using all four methods. Comparison was made with Passing-Bablock regression. The precision of the four methods were assessed using two patient samples of differing concentrations run three times each day for five days (CLSI EP15-A2). Samples taken from patients with Haemoglobin variants were also run on each instrument.

Results

The Abbott Architect enzymatic method and Alere Affinion method demonstrated no significant bias when compared with the Bio-Rad Variant method. The Roche b101 immunoassay method showed a positive bias (average 5%) at lower concentrations. Precision was acceptable on all four platforms. None of the methods were able to produce reliable results from a sample from a Homozygous HbS patient. The Alere, Roche and Architect were all able to demonstrate consistent results with samples from patients with heterozygous HbE and beta thalassemia. The Bio-Rad Variant was unable to produce reliable results from these latter 2 patients.

Conclusion

Our limited study shows acceptable precision and accuracy with the Abbott Architect, Alere Affinion and Abbott enzymatic glycated Haemoglobin assays. The positive bias of the Roche b101 method makes it less suitable. All of the assays have some problems with haemoglobin variants.

P38 CALCULATED OSMOLLALITY – A REFERENCE INTERVAL BASED ON THE AUSSIE NORMAL STUDY

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Introduction

The calculation of serum osmolality from measured analytes is a useful tool in estimating the osmolar gap when it is compared to the measured osmolality. This can help in determining the likelihood of ingestion of low molecular weight substances. It is also useful in the detection of pseudohyponatremia.

Methods

The Aussie Normals study was a large scale a priori study of almost 1800 healthy individuals (ages 20 to 87 years) from the Canberra region with the intention of developing reference intervals with over 80 analytes measured. Patients were excluded based on known disease states (e.g. renal or cardiovascular disease) and some medications. Excluding those samples where glucose had not been measured resulted in 796 results (417 F and 379 M) from which we calculated osmolality using three common formulas. Six low osmolality results were excluded using a 3.5 SD exclusion rule. A comparison was also done using patient samples (varying concentrations of sodium, glucose and urea) to compare the calculated osmolality with the measured osmolality.

Results

Using the Shapiro-Wilk test to determine the reference interval for the simple calculation (2xNa+Glu+K) yielded an interval of 281 to 299 mmol/L, the Weisberg (1975) calculation 272 to 288 mmol/L and the Bhagat et al (1984) calculation a R1 of 280 to 297 mmol/L. Compared to the measured osmolality the Deming regression fit for the simple calculation was 0.92x + 18.3, for the Weisberg Calculation it was 0.87x + 24.4 and for the Bhagat et al calculation it was 0.88x + 22.2.

Conclusion

The study compared well with our current reference range (Bhagat et al) of 280 to 300 mmol/L. At the recent AACB/RCPA Harmonisation Meeting (2015) a consensus was reached to use the simple calculation and this study has demonstrated that there would be little effect from such a change.

P39 MONITORING OF PATIENT RESULT MEDIANS

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Introduction

As part of our internal Quality Assurance System, we have implemented a monitoring system utilising rolling instrument assay means. The Laboratory Information System (LIS) sends email alerts in real time if the cumulative assay mean violates pre-set limits.

Methods

Data was accumulated from 12 Abbott Architect c16000 analysers over a 12 month period. From the average daily median for an assay, limits were set at three standard deviations from this value. Any triggered events were evaluated against the Internal QC program to checks for shifts or trends. Rolling instrument means were automatically calculated every 100 patients and compared with the limits.

Results

Real-time monitoring allowed any relevant shifts in the means to be actioned. Results were not validated until the cause had been investigated. The majority of failures were due either to a run of patients with true abnormal results or a single significantly abnormal patient result. Weekly review of the medians was used to verify consistency between instruments.

Conclusion

We found that monitoring patient results means was a useful addition to internal quality control for our quality assurance system. During a 12 month period, this process detected significant analyte shifts on over 40 occasions. Around 4000 patient repeat analyses were avoided, together with the associated wastage of instrument consumables, and over 80 hours of downtime and troubleshooting was not required.

P40 INCREASING FERRITIN ACROSS NORMAL GGT REFERENCE INTERVALS IN AUSTRALIAN ADULTS – WHAT IS THE TRUE GGT REFERENCE INTERVAL?

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Introduction

As well as an iron storage marker, ferritin is an acute phase reactant, and elevated levels may be seen in conditions such as hepatic steatosis. Due to the increasing prevalence of obesity, finding a “normal” reference population is becoming more difficult, and establishing reference intervals for ferritin may be problematic. We analysed our patient database to assist with establishing ferritin reference intervals, using GGT as an indicator for hepatic steatosis.

Methods

One month of iron studies at SNP where GGT was included, were analysed. Patients were excluded if the following criteria were not met: GGT <100 U/L, ALT <40 U/L, ferritin ≥30 µg/L and ≤600 µg/L. Adult patients (≥ 20 years)
comprising 5668 females and 8418 males met the study criteria. Percentiles were calculated in three groups, the lower and upper halves of the GGT reference interval, as well as from the GGT URL to <100 U/L. Percentile analysis and correlation studies were performed using MS Excel.

**Results**

For females, the ferritin the 95th percentiles were 267 µg/L, 323 µg/L and 364 µg/L for GGT cohorts GGT 5-19 U/L, GGT 20-35 U/L and GGT 36-99 U/L, respectively. For males, the ferritin 95th percentiles were 345 µg/L, 439 µg/L and 499 µg/L for GGT cohorts GGT 5-27 U/L, GGT 28-50 U/L and GGT 51-99 U/L respectively. The average ferritin value rose with increasing GGT, plateauing around a GGT of 15 U/L in females and 25-30 U/L in males.

**Conclusion**

Ferritin levels are significantly increased in patients in the upper half of the GGT reference interval. This suggests that GGT reference intervals are not composed of entirely “normal” individuals – there is likely an upward bias from the inclusion of patients with some degree of fatty liver.

**P42 ASSESSMENT OF THE BECKMAN COULTER ACCESS AMH ASSAY PERFORMANCE AND ESTABLISHMENT OF NORMAL RANGES**

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**Introduction**

Measurement of Total B12 with appropriate reflexing to the more expensive and specific holotranscobalamin (Active B12) assay is an improved strategy to overcome issues of inter-individual variation in transcobalamin I levels that affect Total B12 interpretation. Cut offs for serum Total B12 have been proposed below which Active B12 reflexing should not be performed; the premise being that patients will be truly deficient below this level and therefore do not require an Active B12 performed. We investigated whether this threshold did in fact exist to help reduce the number of Active B12 tests performed.

**Methods**

We analysed our patient database for samples where Total B12 was below 150 pmol/L with corresponding Active B12 levels. A normal Active B12 was considered to be above >35 pmol/L. All serum Total B12 and Active B12 samples were analysed on Abbott Architect i4000 platforms. The lower limit of reporting for Total B12 was 70 pmol/L.

**Results**

We identified 4823 samples with a Total B12 of less than 150 pmol/L. Of these nearly two thirds had an Active B12 level > than 35 pmol/L. Of 50 samples with a Total B12 of less than 70 pmol/L nearly 1 in 6 (8 samples) had an Active B12 of >35 pmol/L.

**Conclusion**

We identified that even below the lower limit of reporting for Total B12 (<70 pmol/L) at least 16% of patients would be incorrectly labelled as deficient if the sample hadn’t been reflexed to Active B12.

**P43 DEFINITIVE REFERENCE INTERVALS FOR TSH ON THE ABBOTT ARCHITECT ANALYZER**

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**Introduction**

The symptoms of thyroid disease are very subtle and many persons presumed to be normal may have some degree of thyroid disorder. This makes defining true reference intervals for TSH difficult.

**Methods**

The AUSSIE NORMALS study enrolled 1856 persons (893 M, 963 F) in good health and performed analysis of >80 analytes from each. In this study 779 subjects (374 M, 414 F) had TSH, fT4, fT3 and thyroid antibodies (anti-Tg and anti-TPO) measured on the Abbott Architect ci16200.

Any persons taking thyro-active medications, or who had either of the thyroid antibodies above the reference interval, were excluded from consideration of their thyroid function test results. One subject was excluded as an outlier using the Dixon method leaving a cohort of 526 (250 F, 276M).

**Results**

The central 95 percent for TSH was distributed between 0.47 and 3.42 mU/L and between 10.6 and 16.4 nmol/L for fT4. The log of TSH and fT4 concentrations was shown to not be significantly different to a Gaussian distribution (p>0.05 and p>0.10 respectively). Similarily the central 95 percent for fT3 was between 3.3 and 5.4 nmol/L and was distributed in a Gaussian manner (p=0.05).

**Conclusion**

In this cohort of healthy persons, and in whom thyroid disease was carefully excluded, the TSH reference interval was lower than usually accepted. The fact that the distribution was Gaussian strongly supports the contention that this was a truly healthy population and that the higher reference intervals often quoted include persons with some degree of thyroid dysfunction.

**P44 A BHATTACHARYA ANALYSIS OF URINE FREE CORTISOL**

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**Introduction**

Cortisol circulates largely bound to cortisol binding globulin (CBG), with a small fraction present as free hormone. Typically less than 2% of circulating cortisol is excreted unchanged in the urine. This fraction, known as urinary free cortisol (UFC), is a particularly useful index of cortisol over-production. Currently Sullivan Nicolaides Pathology reports a single reference interval for both female and male patients.
PREVALENCE OF VITAMIN D DEFICIENCY & HYPOCALCEMIA IN ADULT HEMATOPOIETIC MALIGNANCY

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Introduction
Vitamin D is a steroid hormone with a broad range of biological effects ranging from its classical role as a mediator of calcium and phosphate balance to cellular differentiation and immune modulation. These effects impact normal and dysfunctional hematopoietic and immune function. Vitamin D deficiency prevails in epidemic proportions all over the Indian subcontinent, with a prevalence of 70%–100% in the general population. In India, widely consumed food items such as dairy products are rarely fortified with vitamin D. Indian socioreligious and cultural practices do not facilitate adequate sun exposure, thereby negating potential benefits of plentiful sunshine. Consequently, subclinical vitamin D deficiency is highly prevalent in both urban and rural settings and across all socioeconomic and geographic strata. Aim of this retrospective analysis is to determine the prevalence of vitamin D deficiency in adult patients with haematolymphoid malignancy which may help formulate the policy for vitamin D screening in these patients.

Method
In the year 2014, a total of 91 samples (29 women, 62 men) were received from Adult Haematolymphoid department for measurement of serum vitamin D and serum calcium level as per institutional protocol. All samples were processed on Architect i 1000 by Abbott Healthcare for serum vitamin D and Dimension RXL by Siemens Healthcare for serum calcium level. A biological reference interval of 74.88–99.84 nmol/L for serum vitamin D and 2.125–2.525 mmol/L for serum calcium level was followed.

Results
Out of 91 samples, 63 (69.2%) samples (24 women, 39 men) showed low vitamin D level and 25 (27.4%) samples (5 women, 21 men) showed hypocalcaemia. Prevalence of vitamin D deficiency (69.2%) is comparable with that in the general population (70–100%).

Conclusion
Vitamin D deficiency is common in patients with haematolymphoid malignancy. Considering its role in haematopoietic and immune system processes all patients should be screened for vitamin D deficiency.

INCIDENCE OF HYPONATREMIA AND SYNDROME OF INAPPROPRIATE ANTI-DIURETIC HORMONE SECRETION (SIADH) IN HAEMATOPOIETIC STEM CELL TRANSPLANTS: A RETROSPECTIVE ANALYSIS

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Introduction
One of the most common complications of Haematopoietic Stem Cell Transplant (HSCT) is electrolyte imbalance. Syndrome of Inappropriate Anti-Diuretic Hormone Secretion (SIADH) has also been reported in the literature between 7–13%. We undertook this study to evaluate the incidence of hyponatremia and SIADH in our patients, post-HSCT.

Methods
We analysed 219 (158 male and 61 female; 48 paediatric and 171 adult; 112 allogeneic and 157 autologous transplant) cases of peripheral blood-HSCT conducted between a period of June 2012 to May 2015 retrospectively. Serum electrolyte levels including serum calcium and magnesium were measured daily during the HSCT and subsequently twice to thrice a week during their follow-up OPD visits. Hyponatremia was defined as serum sodium level <136 mmol/L. In hyponatremics further serum and urine osmolality were also carried out.

Results
The incidence of hyponatremia was found to be 9.6% (21/219). Total number of patients with SIADH was 7/219 (3.2%). Incidence of hyponatremia was
9.5% in men, 9.8% in women (p= 0.09385); 12.5% in allogenic, 4.5% in autologous transplant (p= 0.0283); 10.5% in adult and 6.3% in paediatric age groups (p=0.5789) respectively. Serum sodium levels were <= 130 mmol/l in all hyponatraemics. Incidence of SIADH in allogenic and autologous HSCT patients was 5.40% and 0.64% (p=0.022) respectively. Out of all 171 adult and 48 paediatric cases, SIADH was observed in 3.5% and 2% respectively (p=1.0). Incidence of SIADH was 3.8% in men and 1.6% in women (p=0.676). More than 70% of SIADH and 62% of hyponatraemia cases occurred within 100 days post-transplant. Hypocalcemia and hypomagnesemia was observed in 4 out of 7 patients with SIADH.

Conclusion

Incidence of hyponatraemia and SIADH in post-HSCT was observed to be lower in our patients as compared to other published studies. Both Hyponatremia and SIADH were more commonly seen in allogeneic rather than autologous transplant setting.

P49 VITAMIN A AND VITAMIN E DEFICIENCY DEVELOPMENT IN INDONESIA POPULATION

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Introduction

Indonesia has been stated as a vitamin A deficiency country by WHO. Until now, there are only few surveys of vitamin A status in Indonesia, as well as vitamin E. This study evaluated vitamin A and E status based on the role of sex and age in Indonesia population.

Methods

Data was collected from 714 population in Indonesia enrolled in Prodia Clinical Laboratory. Serum retinol and α-tocopherol was measured by HPLC method for vitamin A and E measurement. Normal range was used as cut off for vitamin deficiencies. Normal ranges were 236-911 µg/L and 8-23 mg/L for vitamin A and E respectively.

Results

The mean serum retinol and vitamin E concentration were 521 ± 215 µg/L and 15 ± 5 mg/L. Population with vitamin A and vitamin E deficiency were 4.9% and 2.2%. Based on age group, higher prevalence of vitamin A deficiency were more common in adults than children (5.1% vs 2.0%), whereas vitamin E were the opposite (18.0% vs 1.1%). Both vitamin A and E deficiency prevalences were higher in males (10.5% vs 3.4 %; 7.9% vs 0.7%). In males, adults had higher prevalence of vitamin A deficiency than children (12.8% vs 2.9%), whereas for vitamin E deficiency prevalence was higher in children compare to adults (25.7% vs 2.6%).

Conclusion

The prevalence of vitamin A deficiency in Indonesia has been decreasing for the last decade but still there is 4.9% remain. Moreover, there is 2.2% of vitamin E deficiency in Indonesia, mostly in young boys. Malnutrition is still a health problem in Indonesia that needs better improvement immediately.

P50 DISCORDANT TROPONIN T VS TROPONIN I RESULTS IN PATIENTS WITH SKELETAL MUSCLE DISEASE

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Introduction

Diagnosis of acute coronary syndrome in patients with acute skeletal muscle disease using cardiac biomarkers can be challenging; all will have elevated creatinine kinase (CK) from the skeletal muscle disease process but only some patients have myocardial involvement. Concurrent troponin T (TnT) and troponin I (TnI) measurements may provide critical information.

Methods

We followed 14 patients with elevated CK on whom a reflexed TnT was found to be elevated and repeat testing after a time interval showed no significant change. Further investigations excluded interference in the TnT assay and TnT was measured. Relevant clinical information and laboratory results including muscle biopsy were reviewed to identify possible causes for troponin elevations.

Results

Five patients had no detectable TnI and nine patients had detectable but significantly lower TnI than TnT. Eight patients had a skeletal muscle biopsy result. Patients with detectable TnI included 2 polymyositis, 1 inclusion myositis, 2 inflammatory myositis patients. 4/6 patients with no muscle biopsy were confirmed to be on statin therapy. 3/4 of these patients also had a detectable TnT.

Discussion

A small proportion of patients with skeletal muscle disease have detectable TnI of non-cardiac origin. Discordant troponin results (TnT vs TnI) in patients with skeletal muscle disease may be due to re-expression of TnT in diseased skeletal muscle. When TnI is also detectable, cardiac muscle involvement is likely.

Conclusion

In patients with active skeletal muscle disease TnT may be elevated due to acute coronary syndrome, cardiac myopathy or re-expression of cardiac troponin T in diseased skeletal muscle. In addition to repeat TnT to exclude acute coronary disease, measurement of TnI may be helpful to clarify cardiac involvement.

P51 IS POCT TROPONIN SAFE?

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Introduction

Many sites rely on point of care testing (PoCT) for troponin to risk stratify patients presenting with symptoms suggestive of Acute Coronary Syndrome (ACS). Troponin PoCT is vital for rural and remote sites that do not have timely access to a laboratory. To evaluate if PoCT troponin is safe for the
diagnosis of Acute Myocardial Infarction (AMI), we compared three PoCT assays to a high sensitive laboratory assay on patients presenting to the Flinders Private Hospital with symptoms suggestive of ACS.

Methods
Blood samples drawn at 7 time points post admission were collected from 806 patients. Whole blood samples were immediately tested on the Roche cobas h232 (troponin T) and Radiometer AQT 90 Flex (troponin T and I) PoCT analysers. Bloods were then centrifuged and the plasma samples tested on the Roche elecsys 2010 high sensitive troponin T assay. We analysed the combination of the initial sample and the second sample as it would be performed in a clinical setting. A positive result for troponin was obtained if either sample gave a result above the 99th percentile of the assay.

Results
Results showed thirty-nine patients (4.8%) diagnosed with AMI. Sensitivity, specificity and ROC area under the curve was optimal for the cobas h232 at 12 hours (97%, 93% and 0.950 respectively), at 6 hours for Radiometer troponin T (97%, 96% and 0.967), at 8 hours for Radiometer troponin I (100%, 90% and 0.951) and at 4 hours for high sensitive troponin T (100%, 74% and 0.888).

Conclusion
PoCT troponin can be safely used in the hospital setting for patients presenting with symptoms suggestive of ACS provided clinical pathways with appropriate timing intervals are used. Pathology tests should never be interpreted in isolation and the patient’s clinical picture should always be taken into consideration.

P52 SUMMARY OF BIOLOGICAL VARIATION STUDIES FOR HIGH SENSITIVITY TROPOIN T
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Introduction
Absolute and relative changes in high sensitivity Troponin T (hsTnT) concentration have been advocated to improve specificity of the diagnosis of acute coronary syndrome. Troponin is also recommended for monitoring patients for side effects of clozapine, and other potentially cardiotoxic drugs. What is considered a clinically significant change of hsTnT in such settings is not defined. The reference change value (RCV), derived from biological and analytical variation data, could assist in setting delta change limits that more objectively identify patients at risk of drug-induced myocarditis and which trigger decisions for discontinuation of medication. However, data on biological variation are highly variable and poorly described to enable accurate calculation of RCV.

Methods
We searched for studies published on biological variation using the hsTnT assay. The quality of included studies was evaluated by a recently published critical appraisal checklist, covering the following items: definition of the analyte measured, detailed characteristics of subjects, measurement procedures, study duration, sampling procedures, sample type, conditions of storage, analysis of samples and methods of data analysis.

Results
Within individual biological variation reported in 8 studies ranged from 1.2% (0-6 hours) to 94% (0-8 weeks). There was a large degree of variation in the quality of data reported in published studies, and none included the complete set of recommended checklist items. All studies reported the analytical methodology used, and the number, age and sex, with variable detail on the health/disease status of the subjects involved. Details about statistical techniques were often lacking, and most studies did not refer to a power calculation.

Conclusion
Current biological variation studies for hsTnT lack relevant information needed for accurate comparison and synthesis of published data and translation of the information to meaningful medical decisions in various clinical settings.
P55 CHARACTERISTICS OF INDIVIDUALS WITH ELEVATED TROPTONIN I ON A HIGH SENSITIVITY ASSAY BUT NORMAL LEVELS ON A CONTEMPORARY ASSAY

**Aims**
This study aimed to determine the demographics and clinical characteristics of individuals with elevated troponin I by a high sensitivity troponin I (hs-TnI) assay, but with non-elevated troponin I (Tn-IH) on a contemporary assay. The hs-TnI cut-points for males were ≥26 ng/L and for females ≥16 ng/L.

**Methods**
Individuals with elevated hs-TnI and non-elevated Tn-IH between 18/11/2012 and 24/12/2013 at Royal Perth Hospital were retrospectively reviewed. Clinical characteristics were determined by reviewing the discharge summary for that admission. Males were further segregated into hs-TnI between 26-33 ng/L, and ≥34 ng/L, the internationally proposed male 99th percentile.

**Results**
Of 1449 individuals that had hs-TnI above the gender specific threshold, 120 (8.3%) had Tn-IH levels below the assay specific threshold. There were 77 (64%) females, mean age of 70.3 years. There were 43 (36%) males, 35 had hs-TnI between 26-33 ng/L with a mean age 72.6 years, and eight had levels ≥34 ng/L, mean age of 69.0 years.

There were four males (11%) and four females (5%) diagnosed with acute coronary syndrome. Thirty-three females (42%) had other cardiac diagnoses and forty (52%) had non-cardiac causes. Thirteen males (37%) 26-33 ng/L had other cardiac diagnoses and 18 (52%) had non-cardiac diagnoses. Four males (50%) ≥34 ng/L had other cardiac diagnosis and four (50%) had non-cardiac diagnoses. There were no gender differences in hypertension, diabetes mellitus, congestive cardiac failure, aspirin use or current smoking. There were no significant differences in demographics or characteristics of males with an hs-TnI between 26-33 ng/L and males with an hs-TnI >34 ng/L.

**Conclusion**
A greater proportion of women were identified with elevated hs-TnI and non-elevated Tn-IH between 18/11/2012 and 24/12/2013 at Royal Perth Hospital were retrospectively reviewed. Clinical characteristics were determined by reviewing the discharge summary for that admission. Males were further segregated into hs-TnI between 26-33 ng/L, and ≥34 ng/L, the internationally proposed male 99th percentile.

P56 OUTCOME OF SPECIFIC INTERPRETATIVE COMMENTS SUGGESTING SPECIALIST REFERRAL FOR INDIVIDUALS AT HIGH RISK OF FAMILIAL HYPERCHOLESTEROLEMIA

**Aims**
We sought to determine whether specifically recommending specialist referral increased the clinic referral rate. We considered the addition of the clinic's fax number increased the clinic referral rate.

**Methods**
Individuals with elevated hs-TnI and non-elevated Tn-IH were reviewed. The control group received an interpretative comment suggesting FH as a consideration, whereas the case group received a similar comment that additionally suggested referral to the regional lipid disorders clinic, and a subset of these also received the clinic’s fax number.

**Results**
There were 234 individuals highlighted as being at risk of FH; 96 (42%) were controls and 135 (58%) were cases, of which 99 were also given a fax number. Eight individuals (8%) were referred to the lipid clinic from the control group compared with 21 (16%) from the cases (P=0.04). Amongst the cases that received a fax number 16 were referred (16%), compared with five (14%) in those that did not (P=0.3). After clinic review, four probable and four definite FH individuals were detected in the control group, compared with seven possible, seven probable and seven definite in the case group. All definite FH cases were positive for a LDLR gene mutation.

**Conclusion**
Interpretative commenting augments the detection of FH in primary care. Although specifically recommending specialist referral increased the referral rates compared with raising FH as a consideration, there was no additional benefit of adding a fax number.

P57 IMPACT OF IL-6 GENE ON MALE INFERTILITY

**Introduction**
To investigate the associations of SNPs in the candidate genes IL-6 of the infertile subjects along with assessment of hormone levels and sperm cell death in Indian population.

**Methods**
We undertook genotyping on a total of 640 individuals, including 160 fertile donors as controls and three subgroups of infertile men, normozoospermic (idiopathic unexplained; n=160), oligozoospermic (n=160) and asthenozoospermic (n=160). These participants were selected from Departments of Biochemistry and Urology, K.G’s Medical University, Lucknow, India. We used allele-specific polymerase chain reaction (PCR) and PCR-RFLP to investigate the substitution of the guanine (G)-to-cytosine (C) at position –174 in the promoter regions of the IL-6 genes and their relation to male fertility and sperm function.

**Result**
We found that the substitution level from G to C in the IL-6 genes was significantly higher in oligozoospermic and asthenozoospermic infertile subject groups, respectively. Also, a significant increase in the levels of ROS was observed in both oligozoospermic and asthenozoospermic subjects. In contrast, a significant decrease in the levels of testosterone and luteinizing hormone was observed along with increased prolatin and follicle stimulating hormones of infertile subjects.

**Conclusion**
IL-6 G-174C substitution is strongly associated with male infertility in Indian population. Allele and genotype meta-analysis also supported its strong correlation with male infertility, thus establishing it as a risk factor.

P58 PRO-(IL-18) AND ANTI-(IL-10) PROMOTER GENETIC VARIATIONS AFFECTS SUSCEPTIBILITY AND THEIR SERUM LEVELS IN PROSTATE CARCINOMA

**Introduction**
Inflammation is an important factor of all cancers and Genes of cytokines are subject to polymorphisms in their regulatory regions that may affect the level and ratio of cytokines secreted, thus they may change the inflammation balance of the whole gamut of the body. IL-18, a pro-inflammatory cytokine expressed on numerous cells including prostate gland and main facilitator of immune...
responses with anti-cancerous property while IL-10, an anti-inflammatory
cytokine is associated with tumour malignancy via immune escape. So the
present study hypothesized that IL-18 (pro-) and IL-10 (anti-) inflammatory
genetic variants at -607 C/A, -157G/C and -819C/T, -592C/A respectively may
spawn susceptibility risk and might influence their circulating interleukins
levels in prostate carcinoma (CaP).

Methods
Study includes 582 subjects with 291 prostate carcinoma patients and 291
controls. Genotyping was performed by PCR-RFLP and by real time PCR
probe based method and circulating interleukins level by ELISA.

Results
GG, GC genetic variants and GC>CC mutant genotypes were associated
with a significantly increased risk of prostate cancer (P=0.003, P=0.0001
and P=0.0001 respectively) at -137 promoter of IL-18. IL-18 levels were
significantly elevated with GG at -137 locus and trends observed was
GG>GC>CC while at -607 it varied as CC>CA>AA in CaP and controls. IL-
10 variants at -819 showed IL-10 levels in order of TT>TG>CC (p=0.05) with
TT genotypes, further at -592 locus of IL-10 expression were as CC>CA>AA
in all groups.

Conclusion
Thus current study determines that promoter polymorphism of IL-18 and IL-
10 affects not only susceptibility but also their interleukin levels in prostate
carcinoma.

P59 GENE EXPRESSION OF APOPTOTIC PATHWAY IN
MALE FERTILITY
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Introduction
It is well documented that many factors may impair male fertility including,
endocrine disruptors, genetic and congenital factors, post-testicular
obstruction, vascular abnormalities and anti-spermagenic agents. The local
regulatory control is supported by a large number of cytokines, such as tumor
necrosis factor α (TNF-α) and interleukin-6 (IL-6) Bel-2, Cytochrome C,
Caspase and procaspase.

Objective
To investigate the gene expression IL-6 and TNF α , Bel-2, Cytochrome C,
Caspase and procaspase in infertile subjects for their relationship to sperm
quality and cell death parameters.

Methods
We undertook gene expression on a total of 300 individuals, including
120 fertile donors as controls and three subgroups of infertile men, normozoospermic
(idiopathic unexplained; n=60), oligozoospermic (n=60) and asthenozoospermic
(n=60). These participants were selected from Departments of Urology, K.G’s Medical University, Lucknow, India. We
used quantitative real time PCR (qPCR) with lightCycler Fast Start DNA
PLUS SybrGreen kit for IL-6 and TNF α , Bel-2, Cytochrome C, Caspase and
procaspase mRNA and their relation to male fertility.

Result
We found decreased sperm motion kinetics was associated with decreased
Bel-2 and procaspase expression and increased IL-6, TNF α expression in
oligozoospermic and asthenozoospermic subjects. However, cytochrome
c expression was significantly increased in the oligozoospermic and
asthenozoospermic infertile subjects compared to healthy fertile subjects.

Conclusion
IL-6, TNF-α , Bel-2, Cytochrome C and Caspase gene expression were altered
in men with impaired fertility possibly via their associations with sperm count,
motility and morphology.

P60 DETERMINATION OF A REFERENCE INTERVAL FOR
IGF1 BY DATA MINING USING THE BHATTACHARYA
TECHNIQUE
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Introduction
The Diasorin Liaison XL IGF1 assay was adopted at Sullivan Nicolaides
Pathology (SNP) in 2012 as a replacement for the Immulite IGF1 assay.
Initially SNP continued to use Immulite reference intervals, as the supplied
Diasorin Intervals did not appear a good fit for patient results. After
accumulating sufficient data we performed an analysis of the patient results to
establish a more reliable reference interval.

Methods
Samples collected between September 2012 and December 2013 were assayed
with the Diasorin Liaison XL IGF1 assay; in total 13,179 cases comprising
6,261 males and 6,918 females. Data were analysed by the Bhattacharya
technique and by non-parametric percentiles. Results obtained were compared
with the literature and various manufacturer supplied reference intervals.

Results
Values obtained by Bhattacharya analysis agreed most closely with literature
ranges published by Bidlingmaier et al for the IDS iSYS assay, after adjusting
for correlation between the two assays (JCEM 2014, 99(5):1712). The IDS
IGF1 assay to Diasorin assay correlation used was y = 0.83x - 2.27. For
example, in males ages 50, Bhattacharya 2SD results were 11-31 nmol/L,
compared with adjusted IDS ranges of 10.2-31.9 nmol/L. In this same patient
group, non-parametric analysis indicated 2.5th and 97.5th percentiles of 10
and 42 nmol/L respectively.

Conclusion
Bhattacharya analysis provided useful data for determination of IGF1
reference intervals for the Diasorin Liaison XL IGF1 assay at SNP. The
analysis was validated by comparison with literature ranges published by
Bidlingmaier et al. Non-parametric analysis was not an appropriate guide as
the 97.5 percentile results were biased by a number of patients with elevated
IGF1 levels.

P61 17-HYDROXYPROGESTERONE ASSAYS – TIME FOR
HARMONISATION
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Introduction
Adoption of LC-MS/MS methods for measurement of steroids has
highlighted the need for improved harmonisation of these assays. Following
implementation of an LC-MS/MS method for 17-hydroxyprogesterone (17-
OHep) we realised that adoption of appropriate age and gender related reference
ranges needed to take account of the biases in existing assays seen in quality
assurance programmes and sample exchanges which we have investigated.

Methods
Clinical patient samples were used throughout. We measured 17-OHep by
isotope dilution LC-MS/MS (Shimadzu Nexera LC and AB Sciex 5500
MS/MS; functional sensitivity <0.14 nmol/L) using Isosciences Certimass™
Reference Standard material. Immunoassays were performed by
BM Biomedicals RIA and Immunotech RIA following serum extraction.

Results
The MP Biomedicals immunoassay (n=33) demonstrated a 23% negative bias
to LC-MS/MS while the Immunotech (n=50) showed a 55% positive bias.
Thus Immunotech read 100% higher than MP Biomedicals. How our findings
compare to the soon-to-be-released RCPAQAP Endocrinology Cycle 43 will
be discussed in the poster.

Conclusion
The current lack of harmonisation is highly unsatisfactory but may be
easily remedied by adoption of commutable high level calibrators. In view of
the difficulty of developing age and gender specific reference ranges and
diagnostic cut-offs in children and adults it is critical for the IVD industry to
realign their assays to LC-MS/MS reference methods.
A17-YEAR AUDIT OF TESTING FOR 21-HYDROXYLASE DEFICIENCY
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Introduction
The enzyme 21-hydroxylase (21-OH) in the adrenal cortex catalyses the conversion of 17-hydroxyprogesterone (17-OHP) to 11-deoxycortisol in the cortisol path way and progesterone to 11-deoxycorticosterone in the aldosterone pathway. Deficient 21-OH activity results in reduced efficiency of cortisol production, increased ACTH and diversion of 17-OHP to androstenedione and testosterone. In female infants severe 21-OH deficiency (21-OH) is demonstrated by elevated 17-OHP from birth with marked virilisation while the more common partial deficiency results in mild or unrecognised virilisation. To demonstrate 21-OH deficiency is often necessary to unmask the condition by stimulating 17-OHP production with Synacthen. Until recently reference ranges and cut-offs for screening and diagnosis of 21-OH were defined by immunoassays which are poorly correlated and therefore method-specific. In preparation to changing to LCMS we conducted an audit of all Synacthen tests in patients suspected of 21-OH.

Methods
Baseline and 60-minute post Synacthen 17-OHP were measured by RIA (MP Biomedicals) in 107 patients aged 0-50 years, over a 17-year period.

Results
Post Synacthen 17-OHP results revealed clear separation of normal (all <22 nmol/L, n=95) and 21-OH patients (all >60 nmol/L, n=12), although historically we have defined 21-OH response as >30 nmol/L. There was considerable overlap of the baseline 17-OHP values in normal (0.3-10 nmol/L) and 21-OH patients (2-36 nmol/L).

Conclusion
Diagnosis of 21-OH could not be excluded unless the baseline 17OHP was <2 nmol/L. The baseline and post Synacthen 17-OHP immunoassay results provide a basis for expected results using LC-MS/MS but adjustment will be necessary for the demonstrated method dependant biases.

Evaluation of Roche and Beckman Coulter Automated Anti-Mullerian Hormone Assays
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Introduction
Anti-Mullerian hormone (AMH) is secreted by developing ovarian follicles and mainly used predict ovarian reserve in women. The measurement of AMH has been problematic due a lack of a traceable standard and lack of concordance between methods. The most widely used method, the unmodified DSL AMH Gen II ELISA (Beckman Coulter) was found to be affected by interference from complement and was subject to an urgent medical device recall in 2013. The recent introduction of automated immunoassays by Roche and Beckman Coulter promised to show improved performance compared to the manual ELISA assays. This study compares the performance of the modified DSL Gen II assay with both the automated immunoassays.

Methods
Modified DSL Gen II, Cobas Elecsys E170 (Roche) and Access 2 (Beckman Coulter) instruments were used to run 39 patient sera in parallel. Intra-assay imprecision was determined using 20 replicates of patient sera while inter-assay imprecision was determined by running QC material over 20 days.

Results
P-K regression analysis of patient sera (pmol/L) gave the following equations: Access2 = 0.92 DSL Gen II + 1.35
E170 = 0.75 DSL Gen II + 1.29.
E170 inter-assay imprecision <2.2%CV across range 6.8-37 pmol/L with intra-assay imprecision <1.1%CV across range 9.8-31.9 pmol/L. Access 2 intra-assay <3.6%CV across range 6.3-98.6 pmol/L with inter-assay imprecision <1.6% for range 8.5-16.0 pmol/L.

Evaluation of Roche and Beckman Coulter Automated Anti-Mullerian Hormone Assays
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Development of a Method for the Simultaneous Measurement of Serum Testosterone, 17-Hydroxy Progesterone and Androstenedione by Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS)
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Introduction
Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is ideally suited for accurate, rapid analysis of multiple analytes from a single injection. We developed and validated a sensitive, specific LC-MS/MS method for simultaneous quantitation of testosterone, 17-hydroxyprogesterone (17OHP) and androstenedione. Biochemical assessment of these steroids is important to investigate hyperandrogenism, infertility, polycystic ovarian syndrome, androgen secreting tumours and congenital adrenal hyperplasia. This assay replaced our testosterone immunoassay and by introducing in-house testing for 17OHP and androstenedione, achieved cost-savings for the laboratory.

Methods
After addition of isoelte-labeled international standards, serum samples were deproteinised and extracted with tertiary-methyl-butyli-ether. Supernatants were evaporated and reconstituted. Chromatography was achieved in 15 minutes by RP-C18 column, with a Shimadzu HPLC and ABSciex-QTRAP-5500. The method was validated for linearity, precision, absolute recovery, functional sensitivity, matrix effect and accuracy (against RCPA-QAP target and mean values). Patient correlation studies were performed for testosterone (vs Beckman-Access2), 17OHP, and androstenedione (vs reference LCMS/MS).

Results
Testosterone and androstenedione were linear to 125 nmol/L, 17OHP to 375 nmol/L. Within-run precision for all analytes was less than 5% and between-run less than 8.5%. Absolute recoveries were 69–95%. Functional sensitivity was 0.1 nmol/L for testosterone and androstenedione, 0.3 nmol/L for 17OHP. Matrix effects were minimal. RCPA-QAP and patient sample linearity-regression comparisons (Passing-Bablok) were y = 1.12x+0.12 and y = 1.30x-0.52 (testosterone), y = 1.06x-0.35 and 1.12x+0.01 (17OHP) and y = 1.05x-0.08 and 1.28x-0.24 (androstenedione). Cost analysis indicated significant per annum savings for the laboratory.

Conclusion
This accurate, rapid, sensitive LCMS/MS assay for simultaneous analysis of testosterone, 17OHP and androstenedione compares well with the RCPA-QAP samples and another LCMS/MS method. Replacing the testosterone immunoassay will mean improved precision and accuracy at low levels especially benefitting female, paediatric and oncology patients. Considerable cost-savings will be achieved by ceasing referrals for 17OHP and androstenedione testing.

Review of Sex Hormone Test Requests in a Tertiary Hospital
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Introduction
Sex hormones testing are commonly requested for assessment of the gonadal-pituitary-hypothalamic axis. However, modern methodologies preclude their use especially in the lower end concentration, hence we reviewed our laboratory’s estradiol and testosterone requests to determine the frequency of such results.
Methods
All estradiol and testosterone requests from April 2014 to April 2015 were reviewed, with the first request considered in patients with repeated requests. Estradiol and total testosterone were run on Beckman Coulter Access. Free testosterone was performed using Diassure radioimmunoassay kit.

Results
There were 6771 estradiol requests for 3072 patients, with median age of 33 and 38 years old for female and male patients respectively, and a female: male ratio of 9.1. 178 and 47 patients were below 16 years old or above 65 years old. 289 (93%) male and 1854 (67%) female patients had estradiol levels below the functional sensitivity of 160 pmol/L.

- E2 immunoassays 1st gen Not suitable for postmenopausal women, males, prepubertal/pubertal children
- Testosterone not for children/women

There were 3513 total testosterone requests for 3038 patients, with median age of 31 and 51 years old for female and male patients respectively, with a female: male ratio of 2:1. 90 (10%) male and 196 (9%) female patients had testosterone levels below the detection limit of 0.35 nmol/L, and 171 patients were below 16 years old. There were 200 free testosterone requests for 171 patients, with median age of 30 and 53 years old for female and male patients respectively, with a female: male ratio of 1:16. 3 (2%) male patients had levels below 0.45 pmol/L.

Conclusion
Despite the Endocrine Society’s recommendation that estradiol immunoassays are not suitable for postmenopausal women, males, prepubertal/pubertal children, a large proportion of our laboratory’s estradiol requests came from these groups. Therefore there’s need to communicate with our clinicians about our assay limitations.

P66 PROLACTIN AND REPRODUCTIVE HORMONE STATUS IN OLIGOMENORRHEIC AND INFERTILE FEMALES

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Background
Oligomenorrhea can be a significant problem affecting young women. Oligomenorrhea during the reproductive years may indicate infertility and may cause matrimonial disharmony which is a serious concern in the Asian sub-continent. The present study was designed to assess prolactin, Follicular Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in oligomenorrheic patients in Eastern region of Nepal.

Methods
A total of 126 patients from Department of the Obstetrics and Gynecology with complaints of oligomenorrhea, primary or secondary infertility were enrolled in this study. The immunoassay laboratory of the Department of Biochemistry performed measurement of prolactin, LH and FSH by ELISA method (Eliscan, India). Data were analyzed using SPSS ver. 20, p-value <0.05 was considered significant.

Results
The mean age of patients was 24.3±5.9 (range 15-45 years). Most had oligomenorrhea (76.2%) and 23.8% had primary or secondary infertility (pregnancy was excluded). The oligomenorrheic women had median prolactin 698 mIU/mL (interquartile range 500-919), FSH 8.5 mIU/mL (6.2-15) and LH 6.4 mIU/mL (3.6-15). The infertile female group had median prolactin 784 mIU/mL (516-989), FSH 11.0 mIU/mL (8.1-61) and LH 6.3 mIU/mL (3.5-27). Prolactin and LH values were not significantly different between the oligomenorrhea and infertility group (p=0.043).

Conclusion
Our study found no significant differences in median serum prolactin and LH concentrations between oligomenorrheic and infertile women.
identified at the point of LC-MS analysis enabling data list upload to the instrument. After batch completion quantitation software processed the data ensuring the following parameters were examined, and flagged: Ion Ratios, Internal Standard variation within defined range, QC values within range, Calibrator accuracy, and sample concentration within calibrator range. All results (including flags) from the batch file were then transferred from LC-MS/MS to XQue, where operators were required to action flagged results prior to transfer to the LIS.

Conclusion
A streamlined, integrated data system was established to facilitate data transfer from the point of sample handling to result entry, ensuring sample identification was maintained throughout the process, and results requiring further examination subjected to scrutiny prior to release.

P69 A PRAGMATIC APPROACH TO PAEDIATRIC REFERENCE INTERVALS FOR CEREBROSPLINAL FLUID (CSF) TOTAL PROTEIN, GLUCOSE AND WHITE BLOOD CELL COUNT USING PUBLISHED EVIDENCE, CLINICAL CONSENSUS AND IN-HOUSE VERIFICATION
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Introduction
Few hospital laboratories have enough resources to establish their own paediatric reference intervals. Published reference intervals for cerebrospinal fluid (CSF) total protein, glucose and white blood cell count vary widely between studies in the literature and there is no consensus on the best reference intervals to be used in paediatric populations.

Methods
To establish paediatric CSF reference intervals, we employed a three-phase approach. The literature was reviewed and 25th – 97.5th percentiles were summarized in phase one of the project. During phase two, multiple paediatricians including neurologists, neonatologists and infectious disease specialists were consulted on the most desirable reference intervals to be used based on the literature and their clinical experience. After a consensus on the reference intervals had been achieved, results from at least 20 CSF specimens from each paediatric age group were used to verify the above reference intervals based on both consensus and published evidence. For the purposes of verification, CSF specimens with <200 red blood cells per ul, negative bacterial culture and neither enterovirus nor parvovirus DNA were identified from paediatric patients who also had negative blood and urine bacterial cultures during their hospital admission. Reference intervals were verified based on the methods recommended by the Clinical Laboratory Standards Institute (guideline C28-A3c).

Results
Paediatric CSF reference intervals by consensus were verified as follows.

- Total protein (g/L): 0.3–1.2 (<1 month old); 0.2–0.6 (1 to <3 months old); 0.1–0.4 (≥3 months old).
- Glucose (mmol/L): 2.0–5.6 (<6 months old); 2.4–4.3 (≥6 months old).
- White blood cell count (per ul): 0–20 (<1 month old); 0–10 (1 to <2 months old); 0–5 (≥2 months old).

Conclusion
Using the above approach, we successfully established and implemented paediatric CSF reference intervals that are clinically relevant and locally verified.

P71 THYROID FUNCTION TEST REFERENCE INTERVALS DURING PREGNANCY USING THE VITROS 5600
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Introduction
Harmonization of reference intervals (RI) for thyroid function tests (TFT) is restricted by assay variation, but the derivation of “method-specific” TFT RI has been recommended by professional working parties. This study aims to develop evidence-based, Ortho Clinical Diagnostics (OCD) RI for TSH, fT4 and fT3 in pregnancy, since our laboratory is one of few to report TFT results for this method group in the RCPA QAP Endocrine Program.

Methods
We compared OCD (Vitros 5600) TSH, fT4 and fT3 in pregnant patients with results from Abbott Architect Systems, a frequently used method, to define method-specific variation. The Abbott Architect correlation data and published RI review ‘anchored’ the patient (n=4405) derived, trimester specific RI for OCD TSH, fT4 and fT3 assays.

Results
OCD and Abbott Architect TSH results (0.8–mIU/L) correlated well (r²=0.993), with insignificant bias (y=0.956x – 0.079). Patient data and literature analysis supported 1st trimester (0.09-2.82 mIU/L), 2nd trimester (0.20-2.79 mIU/L) and 3rd trimester (0.31-2.90 mIU/L) RI for OCD TSH.

OCD and Abbott Architect fT4 results (6-20 pmol/L) correlated (r²=0.929), but OCD fT4 values <12 pmol/L showed negative bias (y=0.766x + 3.702). Patient data and literature analysis supported 1st trimester (10.0–25.0 pmol/L), 2nd trimester (9.0–19.0 pmol/L) and 3rd trimester (8.0–19.6 pmol/L) RI for OCD fT4.

There was poor correlation (r²=0.6141) for OCD and Abbott Architect fT3 values between 2-6 pmol/L. Patient data and literature analysis supported 1st trimester (3.5–6.2 pmol/L), 2nd trimester (3.4–5.8 pmol/L) and 3rd trimester (3.3–5.6 pmol/L) RI for OCD fT3.

Conclusion
Abbott Architect correlation data and published TFT RIs ‘anchored’ the review of patient derived, trimester specific RI for OCD TSH, fT4 and fT3. A review of patient data “flagging rates” and clinical consultation confirmed the validity of the derived OCD TSH, fT4 and fT3 RI, prior to implementation.

P72 TOWARDS AGE-RELATED REFERENCE INTERVALS FOR THE HCG STIMULATION TEST IN CHILDREN WITH SUSPECTED DISORDERS OF ANDROGENISATION
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Introduction
The HCG stimulation test is an important investigation to assess testicular function in infants and in children with disorders of sexual differentiation. Although this test has been used over many years, interpretive guidelines, particularly age-related reference intervals are ill-defined. We attempted to establish normal age-related testosterone responses to HCG stimulation.

Methods
An audit of clinical and biochemical pathology records in 70 children given HCG stimulation tests (from 2001 to 2015) at Monash Medical Centre Paediatric Endocrinology Clinic was performed and analysed according to age. Testosterone was measured by immunoassay in 63% of patients and subsequently by isotope dilution LC/MS (LC/MS testosterone = 1.26 x Beckman DxI – 1.16). Dihydrotestosterone (DHT) was measured by RIA.

Results
The 72-hour post HCG testosterone response was highly age related. The upper limit increased from about 15 nmol/L in the first week of life to about 28 nmol/L at 3 months of age and then declined to <10 nmol/L at 2 years until the commencement of puberty when maximal response was >10 nmol/L. The minimal response to HCG in normal patients was more difficult to define due to imprecise establishment of final diagnosis but was <5 nmol/L until onset of puberty.

Conclusion
While these interim results will assist interpretation of HCG stimulation tests this ongoing study will benefit from increasing accumulation of data across the entire paediatric age range and the increased accuracy of testosterone (and DHT) values determined by LCMS. Ideally this would be done by collaboration between laboratories using harmonised LCMS methodology.
P73 PAEDIATRIC REFERENCE INTERVALS FOR IGFI AND IGFBP3 IN SHORT BUT GROWTH HORMONE-REPLETE CHILDREN

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Introduction

Short stature is a common problem presenting to paediatricians with many aetiologies including growth hormone (GH) deficiency in a small minority. Excluding GH deficiency with a single random blood sample is desirable. However in view of the highly pulsatile pattern of GH secretion, random measurements are a highly unreliable test of GH sufficiency. On the other hand IGFI and IGFBP3 are said to reflect average GH levels.

Methods

We used a data mining approach to develop age-related reference intervals for IGFI and IGFBP3 in children who have had GH deficiency excluded by a normal stimulated GH (peak >10 IUL/L) after a fasting exercise stimulation test. 577 children between age 3 and 14 years were included in the analysis. GH, IGFI and IGFBP3 were measured by immunoassay. Outlier IGFI (n=4), GH (n=3), and IGFBP3 (n=3) results were removed by visual inspection of the scatter plots. The remaining results were subjected to LMS (lambda-mu-sigma) analysis with default L, M, S parameters gradually adjusted to fit.

Results

In this group of children, IGFI and IGFBP3 levels gradually increased from age 3 to 14 years. The 2.5%-97.5% percentiles for IGFI increased from 2.0-18.2 mmol/L to 7.7-59.5 mmol/L. The 2.5%-97.5% percentiles for IGFBP3 increased from 22.2-126.5 nmol/L to 72.9-248.4 nmol/L. These intervals were significantly lower compared to the reference intervals in routine use for the general population (IGFI intervals: 6.5-37.5 mmol/L for age 2-5 years, 28.8-127.4 mmol/L for age 13-14 years; IGFBP3 intervals: 35-163 mmol/L for age 3-4 years, 115-348 mmol/L for age 13-14 years).

Conclusion

The age-related IGFI and IGFBP3 reference intervals derived above provide a clue to clinicians to predict children with short stature who are unlikely to be GH deficient.

P74 URINE PROTEIN CREATININE RATIO IN PREGNANCY – PERFORMANCE OF BENZATHIONUM CHLORIDE AND ENZYMATIC CREATININE

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Introduction

Until recently, screening for gestational proteinuria relied on visual interpretation of dip sticks. These have been replaced with automated quantitative methods, and a random urine protein greater than 30 mg per mmol of creatinine is the recommended threshold to initiate further investigation. Urinary protein methods are not standardised, and a study comparing the performance of urine protein methods specifically in pregnant patients has yet to be performed. A pilot study was performed to assess the level of variation in protein measurement as well as urinary creatinine by Jaffe and enzymatic methods.

Methods

Method comparison study with 37 urine samples submitted for routine urine protein creatinine ratio from pregnant women attending a large hospital antenatal service. Urine creatinine was analysed using the Architect modified Jaffe method and Vitros enzymatic creatinine. Urine protein was analysed by benzathionum chloride sourced from two different manufacturers (Abbott and Vitros OCD).

Results

Urinary creatinine by the modified Jaffe and enzymatic methods compared favourably (range 0-25 mmol/L). In contrast the benzathionum chloride precipitation method for urine protein demonstrated significant bias (55%; range 28–76%) tested in range from 0-600 mg/L.

Conclusion

Minimal differences were found with urine creatinines analysed by enzymatic and Jaffe methods in this cohort of patients. However urine protein results differed markedly; at 280 mg/L (decision threshold) the alternative analyser yielded a urine protein between 170–200 mg/L. This discrepancy in protein was surprising and will contribute to non-comparability of patient urine protein in pregnancy as well as the urine protein creatinine ratio. Problems with traceability and accuracy of urine protein are a long standing issue, hence alternative markers such as urine albumin are sought.

P75 EVALUATION OF NEWBORN SCREENING FOR THE DIAGNOSIS OF CARNITINE UPTAKE DEFICIENCY IN INDIAN POPULATION

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Objective

To evaluate the efficacy of newborn screening of free carnitine and short chain acylcarnitines levels in dried blood spots in carnitine uptake deficiency and define optimal cutoff levels using Tandem mass spectrometry in Indian subcontinent.

Materials and Methods- A total of 21,000 dried blood spots of newborns were retrospectively analysed by API 3200ESI/MS/MS instrument.

Results

Eighteen samples were detected as presumptive positive. 13 samples were inconclusive and a second sample was requested. Intra-assay precision for the TMS assay for free carnitine (FC), C2, C3 were FC: QC1-1.71, QC2-2.45; C2:QC1-8.45, QC2-10.24; C3:QC1-5.47, QC2-4.78. All three parameters were individually highly sensitive (99.0%-99.8%) and considerably specific (96%-98.4%). Receiver operating characteristic analysis showed that combining three parameters led to a sensitivity of 99.9% and specificity level of 98.4%. Cutoffs for FC, C2 and C3 were <7.4µM, 7.0µM and <0.2 µM respectively. We also compared our cutoffs with available references for newborn screening and found them to be similar.

Conclusion

Our results suggest that a robust TMS newborn screening with the cutoffs optimized for Indian population will prevent the diagnosis of this highly treatable disease to be missed or misdiagnosed.

P76 IMPLEMENTATION OF PLACENTAL GROWTH FACTOR INTO FIRST TRIMESTER SCREENING FOR DOWN SYNDROME

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Introduction

This study evaluated the benefit of including serum placental growth factor (PIGF) into the existing biochemical combined testing for Down syndrome using free B-human chorionic gonadotrophin (FBhCG) and pregnancy associated plasma protein-A (PAPP-A).

Methods

Random serum samples (87) from patients at 10-13 weeks gestation were measured for PIGF using Thermo Scientific B-R-A-H-M-S Kryptor Auto-analyser. Samples were assayed within 30 minutes of collection. Results were expressed in Multiples of Medians (MoMs) to gestation and correlated to factors specific to the Fetal Medicine Foundation (FMF) program including maternal weight, race, body mass index, maternal age, nuchal translucency and crown-rump length. Samples were classified as Low, Medium or High risk according to Contingency Model (<1/1000, 1/1000-1/1000, >1/1000) guidelines.

Results

Risk using PIGF algorithms were 67/182 (Low/Medium/High) for Contingency Model (range 1/11 to 1/19620, median 1/4137). Addition of PIGF to calculations (range 1/4 to 1/19748, median 1/4032) gave No Change (40), Increased Risk (27, 0.002 to 5.390 magnitude change) and Decreased Risk (20, 0.296 to 10.868 magnitude change). For Contingency Model stratifications No Change was observed in 75 patients, 4 moved from Low to Medium risk, 6 from Medium to Low, and 2 from Medium to High risk.
Conclusion
The use of PI GF in risk calculations for Down Syndrome screening can have an appreciable impact upon the risk in individual cases. When viewed as part of a screening model though, the impact is minimal in terms of overall predictable clinical outcomes with no significant shift in assessment between risk groupings as 20% of patients under the Contingency Model are already being recommended for non-invasive prenatal testing. There is not enough evidence to show at this time that the addition of PI GF significantly improves the screening performance beyond that of the current biochemical testing markers for Down Syndrome.

**P77 HIGH FREQUENCY OF UNDETECTABLE URINE PROTEIN DURING SCREENING FOR GESTATIONAL PROTEINURIA – TRUE LOW PROTEIN OR CLINICAL PROTEINURIA MASKED BY EXCESSIVELY DILUTE URINE?**

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Introduction
Protein creatinine ratio (PCR) greater than 30 mg/mmol in random urine identifies women who need follow up for gestational proteinuria. However urine protein methods have limited sensitivity (LOD 40 mg/L) so a protein concentration reported as ‘undetectable’ may indicate 1. absence of proteinuria or 2. underlying proteinuria masked by excessively dilute urine. We report a high frequency of undetectable urine protein in samples from a large hospital antenatal clinic and identified a small cohort of pregnant women with undetectable urine protein but clinically relevant proteinuria in subsequent tests.

Methods
Urinary creatinine was analysed using the OCD vitros enzymatic creatinine assay. Urinary protein was by benzathonium chloride using Roche reagent.

Results
In 2014, 2979 urine PCRs were requested from 1712 RWH patients. Undetectable protein was reported in 20% of samples (412 women; ¼ of total). 93 women with an undetectable urine protein and follow-up serial PCRs were identified for calculation of PCR intra-individual variation. 70 (75%) were classed as “stable” because subsequent PCR values remained low (<30) with minimal variation. This suggests true absent proteinuria. The other 23 patients (‘proteinuria’ had follow-up PCR > 30 indicating that for a small percentage of women an undetectable urine protein did not preclude an abnormal follow-up PCR. 21 of 23 had urine creatinine <4 mmol/L hence a dilute specimen contributed to masking a clinically relevant proteinuria.

Conclusion
An ‘undetectable urine protein’ logically suggests a significant underlying proteinuria is unlikely. However a 5-fold increase in fluid intake can cause in vivo dilution of urine from a PCR 30 mg/mmol into the ‘undetectable’ range (40 mg/L detection limit is equivalent to 6 mg/mmol). A clinically relevant proteinuria may be disguised. Reflected samples with low urine creatinine to repeat collection will ensure that only valid urine samples are accepted for testing.

Conclusion
The use of PI GF in risk calculations for Down Syndrome screening can have an appreciable impact upon the risk in individual cases. When viewed as part of a screening model though, the impact is minimal in terms of overall predictable clinical outcomes with no significant shift in assessment between risk groupings as 20% of patients under the Contingency Model are already being recommended for non-invasive prenatal testing. There is not enough evidence to show at this time that the addition of PI GF significantly improves the screening performance beyond that of the current biochemical testing markers for Down Syndrome.

**P78 IN VITRO FERTILISATION (IVF) AND FIRST TRIMESTER SCREENING (FTS) FOR DOWN SYNDROME**

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Introduction
First Trimester Screening (FTS) measures Nuchal Translucency, Free Beta Human Chorionic Gonadotropin (Free βhCG), Pregnancy Associated Plasma Protein A (PAPP-A) and Placental Growth Factor (PlGF). Biochemical markers may be altered following In Vitro Fertilisation (IVF). Study aims were to compare levels of Free βhCG and PAPP-A in non-IVF and IVF pregnancies as well as to compare pregnancies using fresh or frozen embryos.

**Methods**
During June 2013 to April 2015, 16187 non-IVF and 343 IVF pregnancies were measured for Free βhCG and PAPP-A using immunoassay (Roche Elecsys). PI GF was omitted due to insufficient IVF pregnancies as PI GF was introduced later. Separate medians were calculated and 10,11,12 and 13 weeks for non-IVF and IVF pregnancies, allowing a comparison of Multiple of Medians (MoM). Levels were also compared in pregnancies using fresh or frozen embryos.

**Results**
IVF pregnancies constituted (2.1%) of FTS all requests. Fresh (69) and frozen (83) embryos were used. In 191 this information was not provided. Levels of PAPP-A and Free βhCG increased and decreased respectively in non-IVF compared to IVF pregnancies. Separate median regression analysis showed similar changes from 10 to 14 weeks for both markers, where the calculated MoMs were similar in non-IVF compared to IVF pregnancies. Percentages using fresh embryos had FTS significantly earlier (p<0.01) compared to pregnancies using frozen embryos. PAPP-A levels were lower (p<0.01) in pregnancies using fresh compared to frozen embryos but unchanged for Free βhCG.

**Conclusion**
IVF pregnancies constitute a significant component of FTS. Minimal change in MoM data and subsequent risk assessments were observed when pregnancies are corrected for IVF. Higher levels of PAPP-A, measured in this small dataset in pregnancies where frozen embryos were used would result in an overall lower risk for aneuploidy. Further studies, validating this finding will allow for an appropriate correction for PAPP-A.

**P79 A TRAINING AND COMPETENCY ASSESSMENT PROGRAM AT RCPAQAP**

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Introduction
Online delivery of training and competency assessment plays an integral part of development activities and has advantages over in-person training as it can be delivered to all staff anytime, and is cost-effective and efficient. RCPAQAP built modules aimed at medical scientists and launched five modules in 2015 with support from the Department of Health.

**Methods**
A learning management system (LMS) was utilised to manage the online content and allow customers to enrol. The LMS needed training manager functionality, where managers could monitor staff training progress. Subject matter experts were identified and engaged to produce content for the modules. Professional development points were assigned by relevant professional bodies.

**Results**
Five modules included Quality Assurance and Quality Control, Snake bite: the role of the pathology laboratory, Select safe sites for venepuncture and two Cytopathology modules. All modules were awarded the highest possible professional development points. Feedback indicated the modules are easy to use, relevant and informative.

**Conclusion**
The modules have been well received by laboratory staff and development continues on a Haematology Morphology course and discussions are ongoing for the development on other disciplines such as Transfusion, Microbiology and Serology.
P80 SHOULD WE TARGET SET OUR RCPAQAP MATERIAL when there are gaps in the traceability chain? Target assignment of 17-OHP, androstenedione, DHEA and DHT by GC-MS/MS

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Introduction
Participation in an external quality assurance program is a central pillar supporting method harmonisation. Assignment of target values is preferable to participant medians. However, reference materials and methods are not available for all analytes including a number of steroids. We therefore proposed to evaluate the establishment of an independent target value for serum steroids by GC-MS/MS.

Method
Four serum steroids 17-hydroxy progesterone (17-OHP), androstenedione, DHEA and dihydrotestosterone (DHT), reported in the RCPAQAP Endocrine Program with median values were assessed. The measurement procedure for these target were based on isotope dilution GC-MS/MS. The calibrators generated by the LC-MS/MS and immunoassay participant results for cycle 43.

Results
Target values for the six levels of QAP material for 17-OHP, androstenedione, DHEA and DHT were developed and assessed. Results for each analyte generated by the LC-MS/MS method participants were within the Allowable Limits of Performance when compared to the target values; with 17-OHP demonstrating a lower recovery by GC-MS/MS compared to LC-MS/MS. For 17-OHP and DHEA the immunoassay results fell into two groups; one showing a negative bias compared to the target and the other demonstrating a positive bias. For androstenedione and DHT all the immunoassay results demonstrated a positive bias compared to the LC-MS/MS and target values assigned.

Conclusion
The utilisation of these independent GC-MS/MS values as targets provides confirmation to the LC-MS/MS minority of participants in the Endocrine Program. They also clearly demonstrate the discrepancy between immunoassay manufacturers’ results for 17-OHP and DHEA and positive bias of immunoassays for androstenedione and DHT. Ideally this GC-MS/MS method will be submitted to the Joint Commission for Traceability in Laboratory Medicine as a pillar in the harmonisation and eventual standardisation of these analytes.

P81 ESTABLISHMENT OF AN EXTERNAL QUALITY ASSURANCE PROGRAM FOR SERUM DIHYDROTESTOSTERONE.

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Introduction
Serum dihydrotestosterone (DHT) is an important analyte for the clinical assessment of disorders of sexual development (DSD). It is also reportedly a difficult analyte to measure. Currently there are significant gaps in the standardisation of this analyte with no certified reference material, method or laboratory recognised by the Joint Commission for Traceability in Laboratory Medicine. In addition, there is no external quality assurance (EQA) program available worldwide to allow for peer review performance of DHT. We therefore proposed to establish a pilot EQA program for serum DHT.

Method
The 2015 RCPAQAP Endocrine program material was assessed by one laboratory prior to the commencement of the pilot program to determine if DHT was present in the material. Target values for the material were provided by the Steroid Research & Mass Spectrometry Unit of the Laboratory for Translational Hormone Analytics in Pediatric Endocrinology at the Justus Liebig University in Giessen, Germany using a measurement procedure based on isotope dilution GC-MS/MS. An isotopically-labelled analogue of DHT (16,16,17-d3-DHT) was used as an internal standard and the DHT calibrator values were based on weighed values of pure DHT material (>97,5% purity) from Sigma (St. Louis, US). The allowable limits of performance (ALP) were established as +/-0.1 up to 0.5 nmol/L and +/-15% for targets >0.5 nmol/L.

Results
Target values for the six levels of QAP material for DHT were established by GC-MS/MS, ranging from 0.02 to 0.43 nmol/L. The material demonstrated linearity across the six levels with a best fit polynomial regression of y=0.002846+1.024x. There were four participating laboratories for this pilot study. Results of the LC-MS/MS methods were within the ALP when compared to the target values; whereas the results from the immunoassay methods were consistently higher than the target values and outside the ALP.

Conclusion
This report provides the first peer comparison of serum DHT measured by mass spectrometry and immunoassay laboratories. Establishment of an EQA program provides one of the pillars to achieve harmonisation / standardisation of the method.
P83 A SIMPLE TOOL TO IDENTIFY AT RISK ASSAYS USING EQA DATA
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Introduction
Maintaining the quality of clinical assays continues to challenge pathology laboratories despite improvement in data handling and analyser capabilities. To achieve commutable and precise results requires suppliers providing appropriate calibrators, an effective laboratory QC system and careful interpretation of External Quality Assurance (EQA) results which identify if the laboratory or analyser method group perform poorly compared to peers and achievable best practice. Clinical laboratories must also have targeted processes in place to reduce patient risk, including risk from poorly performing assays.

Methods
Risk is a function of the clinical application of a test result in patient diagnosis or monitoring and is therefore dependent on the frequency of errors in test results and reliability of the QC system in identifying those errors. The tool relies on three concepts: performance based on Assay Capability, a 3 x 3 matrix to grade peer performance, and calculation of a risk score based on analytical reliability and performance compared to biological variation targets.

Results
We demonstrate the value of the approach by calculating risk for ten common clinical chemistry tests we determined risk using recent EQA results.

Conclusion
We have suggested an objective procedure for laboratories to identify poorly performing assays when compared to their peers and a method of determining risk based only on performance in an EQA. A limitation of using EQA performance is the potential lack of commutability. However performance in EQA is generally stable and as all laboratories are analysing the same sample it does represent a reasonable sampling process. The analysis also uses a calculated capability index, so as long as the EQA uses the same material during the survey estimates of imprecision should be valid.

P84 END OF AN ERA – DECOMMISSIONING THE ABBOTT TDxFLx FOLLOWING THE ASSESSMENT OF TWO CHEMILUMINESCENT METHOTREXATE IMMUNOASSAYS
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Introduction
Fluorescence polarization assays (FPIA) for methotrexate (MTx) have been used since the 1980s to monitor high dose MTX toxicity and assess the need for “rescue” leucovorin therapy in neoplastic patients. Recently, two MTx chemiluminescent immunoassays (Abbott Diagnostics and ARK Diagnostics) have been developed. We assessed the assay performance of each method and the clinical comparability of patient MTx results, to enable decommissioning of the Abbott TDxFxLx analyser in our laboratory.

Methods
Methotrexate levels (0.05 – 60 µmol/L) were measured in neat and diluted (1:11 and 1:201) patient serum samples. Initially, patient (n=19) results were compared to results from the Abbott Architect i1000 (ARK) and ARK (Roche Cobas 6000) assays. We assessed the assay performance of each method and the clinical comparability of patient MTx results, to enable decommissioning of the Abbott TDxFxLx analyser in our laboratory.

Results
Over the analytical range tested, the Architect i1000 levels had a higher probability of association with TDxFxLx values, when compared to those from the Roche Cobas 6000 (p = 0.13). This was particularly evident in patient samples assayed neat or 1:11 diluted, with values < 1.50 µmol/L (Abbott: p = 0.39, ARK: p = 0.11). Patient MTx levels on the Architect i1000 were highly correlated (r=0.998), without significant bias (y=1.0542x - 0.0496) from TDxFxLx values. At an MTx concentration of 0.44 µmol/L, the intra- and inter-run CVs for the Architect i1000 method were 1.32% and 1.54%, respectively.

Conclusion
Abbott and ARK Diagnostic assays demonstrated satisfactory performance for monitoring toxicity in high-dose MTx regimens in our initial evaluation. The Abbott Architect MTx results, including those < 0.40 µmol/L, were linearly related to TDxFxLx MTx levels. This assay also demonstrated low intra- and inter-run CVs and was implemented at the Royal Children’s Hospital to enable decommissioning of the Abbott TDxFxLx analyser.

P85 HOW LOW CAN YOU GO? AN EVALUATION OF THE NEW ABBOTT ARCHITECT ci2000 IMMUNOASSAY IN THE THERAPEUTIC DRUG MONITORING OF METHOTREXATE
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Introduction
Methotrexate (MTx) is a cytotoxic folate antimetabolite used in high-doses for the treatment of some cancers and in low-doses for non-malignant conditions including rheumatoid arthritis. In cancer, MTx monitoring at very low levels is essential to avoid toxicity and to determine when leucovorin administration should commence. The aim of this project was to compare the performance of the Abbott Architect ci2000 MTx immunoassay (AAM) against the current assay utilising ARK Diagnostics reagents (ARK) on the Roche Cobas 502 analyser.

Methods
Imprecision, linearity and analytical sensitivity were assessed for both assays. Forty specimens from patients undergoing MTx therapeutic drug monitoring (TDM) were run in parallel on AAM and ARK as a method comparison. Linearity and analytical sensitivity were assessed using MTx spiked into MTX-free serum.

Results
The AAM demonstrated good precision at three levels, with within-batch precision CV’s of 4.4, 2.4 and 3.2% and between-batch CV’s of 6, 5 and 4% at levels of 0.04, 0.07 and 0.45µmol/L respectively. This compares with historical ARK data between-batch CV’s of 9, 6.8 and 4.5% at levels of 0.04, 0.07 and 0.8µmol/L. Both assays showed good linearity as assessed using dilutions of MTX-spiked serum (AAM: y = 1.133x + 0.0007, R² = 0.9985; ARK: y = 1.3076x - 0.0109, R² = 0.9886). The functional sensitivity was shown to be at least 0.012µmol/L for AAM which compares well with the manufacturers’ limit of detection claim of 0.009µmol/L. Deeming data analysis showed good correlation between the two methods. However, AAM was significantly lower than ARK – particularly at low concentrations, AAM = (-0.01 + 0.9ARK) for concentrations 0.012- 0.748µmol/L and AAM = (-0.01 + 0.75ARK) for 0.012-0.095µmol/L.

Conclusion
The AAM method demonstrated superior precision, linearity and sensitivity at low concentrations around clinical decision points and is a suitable alternative to the ARK reagents for MTX TDM.

P86 RAPID AND EFFICIENT ENZYMATIC HYDROLYSIS OF CODEINE AND MORPHINE GLUCURONIDES IN URINE
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Introduction
LCMS procedures for the confirmation of drugs of abuse in urine are now used in most laboratories, because multiple drugs can be assayed at the same time and sample processing is quick and easy, resulting in faster turnaround times. For opiates analysis, hydrolysis of the glucuronide bonds is required and enzymatic hydrolysis is the preferred option. However, up to date the yields of codeine from the enzymes used have been relatively low even with long incubation times, as the codeine 6 glucuronide bond is difficult to cleave. This study examines the Kura BG 100 enzyme and Patella Vulgata to breakdown the glucuronide bonds of codeine and morphine under various incubation conditions.
**Methods**

Enzymatic hydrolysis was performed on urine spiked with codeine and morphine glucuronides over the range 500-10000 µg/L and codeine and morphine concentrations determined by LCMS. These results and results from authentic urines were compared to acid hydrolysis and assayed by GCMS.

**Results**

The Kura enzyme BG 100 (Halotis Rufescens) obtained from abalone gave recoveries of codeine from urine spiked with codeine 6 glucuronide of greater than 90%. Morphine recoveries for both the 3 and 6 glucuronides were similar. Incubation was carried out at 68 degrees C for one hour using an enzyme concentration of 20000 units per ml of urine.

Results for non- spiked urine specimens in the Austox Quality Assurance Program and authentic patient urine specimens from subjects on codeine medication have shown an acid hydrolysis to Kura enzyme ratio of 0.95 for codeine and 1.27 for morphine, indicating significant agreement.

**Conclusion**

Since high yields of codeine and morphine can be obtained with a relatively short incubation time, the Kura enzyme is ideal for the hydrolysis of opiates, and can be used when assaying for multiple drugs in the same run using LCMS.

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**P87 AN EVALUATION OF THE ABBOTT ARCHITECT ASSAY AGAINST THE ABBOTT TDX METHOTREXATE ASSAY**

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**Introduction**

The long awaited replacement for the Abbott TDX Methotrexate assay is now on the Architect. We evaluated its performance against the TDX assays as it has been the most widely used method. The improved performance at low concentrations and linear measuring range after dilution are significant improvements. The non-toxic state is regarded as below 0.5 µmol/L / 0.2 µg/mL and methods must be able to perform accurately below this range to discharge patients.

**Methods**

Sixty three patient samples were compared and the results tested for concordance, linearity, blank and minimal detection concentration. The performance of QAP samples were also compared with the expected mean result for QAP samples.

**Results**

The concordance correlation coefficient between the 63 samples was 0.9974, with a 95% confidence interval of 0.9967-0.9979. The Pearson Precision Coefficient was 0.9996 and the bias correction factor (for accuracy) was 0.9978. Passing Bablok regression (R² 0.9991) yielded similar results with an intercept of 0.004 (95% C.I. 0.0013-0.001) and a slope of 0.95 (C.I. 0.93-0.97).

The standard deviation for the two populations was 1.5 Architect and 1.4 TDX with median values of 0.18 and 0.16 respectively. There was a slight positive bias in the high range (9-10 µmol/L, 4.1-4.5 µg/mL) of 7%. The bias was not present in the lower range and only 6 results out of 63 differed between assays by greater than 0.1. Cu-sum analysis also confirmed linearity between results.

The lowest detected patient value was 0 compared with <0.2 / <0.09 µmol/L, µg/mL for the TDX. The QAP data (n=39) at three levels gave 0.033±0.001, 0.21±0.009 and 0.47±0.037 for the Architect and 0.035±0.002, 0.2±0.014, 0.47±0.07 for the TDX against target values of 0.03, 0.20, 0.45 µg/mL.

**Conclusion**

The Architect assay measured accurately at a much lower range than the TDX assay and did not show the bias at low results that affect patient discharge for the TDX and the Ark assay. The linear concentration range of the assay was significantly with dilutions was excellent and the slight bias between high values may be due to different abilities of the assays to detect drug metabolites.

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**P88 LC-MSMS ALLOWS DETECTION AND MONITORING OF ANTI-PsYCHOTIC POLYPHARMACY**

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**Introduction**

In psychiatric practice, antipsychotic polypharmacy (APP) is common with a variable incidence around the world. APP can lead to greater drug side effects, often without improving clinical outcomes. Although therapeutic drug monitoring (TDM) is part of clinical protocols for clozapine, other atypical neuroleptics are not usually measured. The aim of this study was to utilise a new in-house LC-MSMS profiling method to determine the frequency and type of APP in our clozapine-patient population.

**Methods**

Plasma (50 µL) was mixed with stable iso-otope-labelled internal standards prior to precipitation and removal of proteins with acetonitrile. Extracts were injected onto a Kinetex Biphenyl core-shell HPLC column to separate 9 antipsychotic drugs and metabolites, which were detected and quantitated by tandem mass spectrometry using highly specific mass transitions after electrospray ionization. The run time was 6 min per sample.

**Results**

From 331 patients undergoing clozapine TDM over one month, APP was present in 37%, with 30% having one, 6% having two and 1% having three or four atypical neuroleptics in their plasma in addition to clozapine. The most frequent extra antipsychotics, and their plasma concentration ranges, were paliperidone (19%; 5-161 µg/L) and aripiprazole (11%; 6-135 µg/L), while olanzapine (7%; 5-57 µg/L), quetiapine (6%; 7-1171 µg/L) and risperidone (4%; 5-29 µg/L) were also found. Compared to clozapine monotherapy patients, APP patients were of similar age (median 38 y) and gender (70 vs 66% male). They had a higher proportion of plasma concentrations below the clozapine therapeutic range (40 vs 29%, p<0.05), possibly because of incomplete cross-tapering from clozapine to other neuroleptics or drug interactions, with similar proportions (33%) above the clozapine therapeutic range.

**Conclusion**

LC-MSMS profiling of atypical antipsychotic drugs could allow a more comprehensive TDM service at no extra cost for psychiatric patients in whom APP is frequent.

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**P89 LABORATORY EVALUATION OF FOUR DRUGS OF ABUSE IMMUNOASSAY KITS**

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**Introduction**

Immunoassay kits require laboratory evaluation to assess their performance and whether they are suitable as screening tests for the target compounds with respect to the cut off concentrations recommended by the manufacturer. This study examines four Thermostifer immunoassay kits for barbiturates, phencyclidine, oxycodone and propoxyphene in urine.

**Methods**

Samples were prepared by spiking blank urine with the drugs of interest at the cut off and at concentrations 25% above and below the cut off to determine recovery and accuracy. Ten samples were prepared for each drug concentration (total of 40 samples for each drug). Precision was also determined by performing analysis of supplied low and high quality control material (n=10). All assays were run on the AU5810-06 analyser after calibration according to the manufacturer’s inserts.

**Results**

There were no false positive or false negative results. All assays apart from phencyclidine marginally over recovered, with recoveries decreasing as the concentration increased. At the cut off concentration, recoveries for barbiturates, oxycodone and propoxyphene were higher than the target concentration by no more than 12%. For phencyclidine, recoveries were 7% lower at the cut off concentration. The coefficient of variation for all drugs over all concentrations was less than 5%. When the manufacturer’s low and high
controls were assayed, all drugs apart from propoxyphene under recovered (but by less than 10%). The propoxyphene low control under recovered by 12%. The coefficients of variation for low and high controls of all drugs were less than 7%.

**Conclusion**

The four immunoassay kits evaluated performed satisfactorily and are suitable as screening tests. Furthermore, these kits have excellent precision around the cut off concentrations recommended by the manufacturer.
<table>
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<th>Author</th>
<th>Presentation</th>
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</thead>
<tbody>
<tr>
<td>Al-Hinti, M</td>
<td>P87</td>
<td>S48</td>
<td>Chavan, P</td>
<td>P47</td>
<td>S36</td>
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<td>S36</td>
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