viapath

On a journey to transform pathology services

Scientific Innovation at Viapath

IBMS Congress 2015

A collection of some of our scientific and academic output from 2015

Who are we?

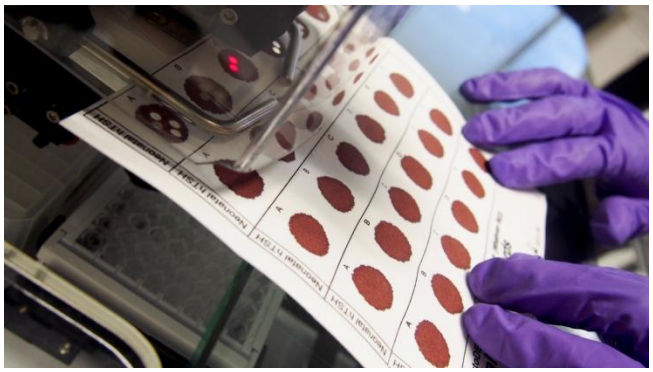
We are Viapath, the UK's leading independent pathology services provider.

Majority owned by the NHS but with the commercial freedom to invest in innovation, we are on a mission to transform pathology services in the UK.

Find out more

Read on for some of our people's latest innovations which have been presented at conferences throughout the year.

Find out more about Viapath at our website www.viapath.co.uk



What we do

As well as operating a world class pathology operation from over 60 laboratories in London and Bedford, we are also a member of the renowned King's Health Partners Academic Health Sciences Centre.

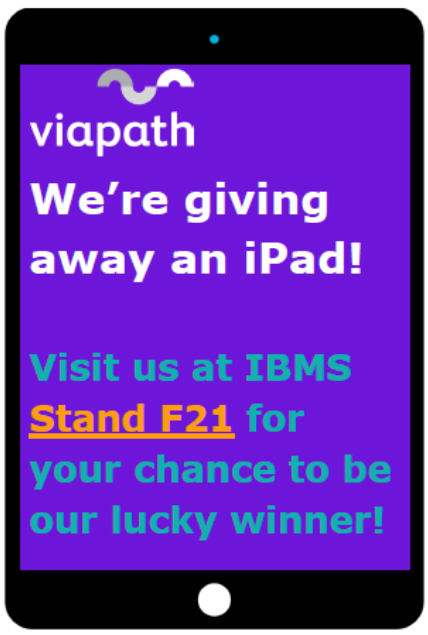
More than just a pathology service, our experts routinely collaborate on projects, bringing together some of the world's brightest scientific minds to advance diagnostic medicine for the benefit of our patients.

Innovation and quality are at the core of everything we do.

IBMS Congress 2015

Tuesday 29 September	The growing need for a more analytical approach to specimen dissection **Dr Guy Orchard**	Cellular Pathology **09.00 – 09.30**
	Specialist portfolio in cellular pathology – an advice and guidance session **Dr Guy Orchard**	Cellular Pathology **13.00 – 14.00**
	Vitamin K metabolism and action of warfarin **Mr David Card**	Haematology **14.30 – 15.00**
	Field aspects of haemoglobinopathy diagnostics **Dr Yvonne Daniel**	Haematology **15.00 – 15.30**
	Diploma of expert practice in Mohs techniques **Dr Guy Orchard**	Cellular Pathology **16.00 – 16.30**
	Non-standard platelet assays **Mr James Maloney**	Haematology **16.30 – 17.00**
Wednesday 30 September	When Haemostasis assays mislead **Dr Gary Moore**	Haematology **09.30 – 10.00**
	Pharmacogenetic testing to personalise cancer chemotherapy **Dr Anthony Marinaki**	Clinical Chemistry **10.30 – 11.00**

Listen to fascinating presentations and discussions from our people and visit us in the IBMS exhibition at **STAND F21** for your chance to win an iPad!



Development of new and accurate measurement devices (TruSlice and TruSlice Digital) for use in histological dissection: an attempt to improve specimen dissection precision

Histological dissection of human tissue has relied on conventional procedures, which have largely remained unchanged for decades. Practices to determine measurement parameters employed in these procedures have largely relied on the use of rulers and weighing scales. It is well documented in the scientific literature that both fixation and processing of tissue can significantly affect the viability of the of tissue sections both for tinctorial and immunocytochemical investigations. Both of these factors can be compounded in their negative effects by inappropriate sampling of tissue at histological cut up. There are five key factors to ensure good surgical grossing technique, flat uniformly perpendicular specimen cutting face, appropriate immobilisation of the tissue specimen during grossing, good visualisation of the cutting tissue face, sharp cutting knives and the grossing knife action. Meeting these factors implies the devices are fit for purpose. Here we describe an innovative approach to designing cut up devices to improve accuracy and precision, which take these five key requirements into consideration.

The devices showed accuracy and precision, enabling tissue slices to be produced in a uniformly perpendicular fashion to within 2 mm in thickness and to enable consistency and reproducibility of performance across a series of tissue types. The application of a digital rule on one of these devices ensures accuracy and also enables quality control issues to be clearly assessed. As cellular pathology laboratories conform to ever increasing standards of compliance and performance in practice, the advent of assured precision and accuracy at cut up is awaited. Recommendations from accreditation bodies such as the United Kingdom Accreditation Service (UKAS) continue to push for improvements in this area of histological investigation. These newly designed devices may give the answers to these requirements and provide the impetus for a new generation of innovative equipment for histological dissection.

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Pan-cytokeratin markers for rapid frozen section immunocytochemistry from head and facial Mohs cases of basal cell carcinoma: a comparison and evaluation to determine the marker of choice

The application of immunocytochemistry in the field of Mohs micrographic surgery (MMS) is well established. This study evaluates the use of pan-cytokeratins (AE1/AE3, MNF116 and AE1/AE3+PCK26) in the assessment of basal cell carcinoma (BCC) on frozen tissue debulk specimens. Fifty-five cases of BCC, all from head and facial sites, were assessed in the study. In addition to staining all cases for the three cytokeratin antibodies under investigation, sections were also stained with haematoxylin and eosin (H&E) to demonstrate tumour architecture and morphology. All sections for immunocytochemistry were stained on a Roche Ventana BenchMark Ultra automated platform employing a rapid frozen section protocol. Results were assessed based on the intensity of staining of keratinocytes (scale: 0–100%), as well as sensitivity of staining determined by the total percentage of keratinocytes stained within the tissue section. AE1/AE3 demonstrated the most consistent staining both in terms of intensity of staining and sensitivity, with a mean of 99.1% and 99.9%, respectively. AE1/AE3+PCK26 average results indicated scores of 70.6% for intensity and 87.2% for sensitivity, with MNF116 scoring 92.9% for intensity but only 57.3% for sensitivity.

The data indicate that AE1/AE3 is the best pan-cytokeratin antibody to use in the assessment of BCC in MMS. The use of cytokeratin immunocytochemistry is justified in morphologically complex cases of BCC, or in cases where dense inflammatory infiltrate surrounding any suspicious cells make identification of small numbers of tumour cells difficult to determine with just an H&E stain. The significant rationale is that cytokeratin staining is a valuable adjunct in the study of tumour cell assessment in cases of MMS for BCC. In addition, the use of anti-AE1/AE3 cytokeratin antibodies provides the most consistent staining results for such cases.

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Automation of Technozym® ADAMTS-13 assays on a Dynex DS2 analyser

ADAMTS-13 is a metalloprotease that cleaves large von Willebrand factor (VWF) multimers into smaller, less reactive multimers. Congenital or acquired deficiency of ADAMTS-13 leads to the potentially lethal syndrome of thrombotic thrombocytopenic purpura (TTP). Acquired TTP is usually due to autoantibody development, or can arise from massive endothelial activation releasing large amounts of ultra-large VWF multimers sufficient to overwhelm degradation capacity of ADAMTS-13. The Technozym® ADAMTS-13 activity and inhibitor assays (Pathway Diagnostics, Dorking, UK) are chromogenic assays employed to detect deficiency of ADAMTS-13 and the presence of an inhibitor to distinguish between congenital or acquired TTP.

Protocols were written and optimised for both assays on a Dynex DS2 ELISA analyser (Werfen Group UK, Warrington, UK) in line with the manufacturer's assay performance instructions. Intra-assay precision for ADAMTS-13 activity from a normal control sample (n=10) gave a CV of 0.02%. Inter-assay precision CVs from normal and low controls (n=4) were 4.0% and 7.0% respectively. Intra-assay precision for ADAMTS-13 inhibitor from a calibrator diluted to a moderately elevated level and normal level analysed ten times gave CVs of 2.47% and 0.15% respectively. Inter-assay precision CVs from normal and high controls (n=3) were 0.08% and 0.03% respectively.

ADAMTS-13 activity reference range derived from 2.5th – 97.5th percentiles of results from 55 normal donors was 66.4 – 107.9%. In view of similarities between the manufacturer's cut-offs for ADAMTS-13 inhibitor (n=193) and locally derived values (n=39), they were merged to give the following cut-offs for diagnostic testing: Negative: <10 U/mL, Borderline: 10 – 15 U/mL, Positive: >15 U/mL.

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Mitochondrial DNA depletion syndrome: to transplant or not to transplant? A case of neonatal DGUOK deficiency

Mitochondrial DNA (mtDNA) depletion syndromes (MDDS) are rare autosomal recessive inherited metabolic disorders that result from a reduction in mtDNA.

Manifestations include; myopathic, hepatocerebral, encephalomyopathy and cerebrorenal disease. Diagnosis involves genetic testing for mutations in nuclear genes responsible for mtDNA maintenance (e.g. TK2, DGUOK, MPV17 and POLG). Liver transplantation maybe controversial if the patient is not neurologically intact.

We report a case of neonatal liver failure in a 1-month-old girl born to consanguineous parents referred for liver transplant assessment. She presented with IUGR, hypoglycaemia, jaundice, lactic acidosis and a coagulopathy; her newborn screen showed raised tyrosine, phenylalanine and methionine. Although succinyl actetone was found to be normal, tyrosine, lactate, AFP and ferritin remained raised.

Liver ultrasound was mildly fatty and initial ophthalmology was normal, but nystagmus and developmental delay soon developed raising the suspicion of a MDDS. Muscle biopsy was unremarkable but, genetic testing revealed a homozygous deletion of four nucleotides in the deoxyguanokinase gene (DGUOK) carried in both parents, confirming a MDDS – 20 % of which are due to hepatocerebral disease.

Visual evoked potentials showed interhemispheric asymmetry suggesting right-sided retrochiasmal dysfunction and, as neurological manifestations are contraindications to liver transplantation this was not pursued ; she later died at 4-months of age.

We review the multidisciplinary diagnostic investigation of MDDS, to enable the challenging decision to transplant or not, providing counselling for families and supportive therapy for patients.

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A four year review of a porphyria genetics centre in the UK

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Introduction

The porphyrias are a group of mainly inherited diseases affecting the haem synthesis pathway that can be divided into two groups based on their clinical symptoms. The acute porphyrias include acute intermittent porphyria (AIP), hereditary coproporphyria (HCP) and variegate porphyria (VP). The biochemical hallmark of an acute porphyria attack is the increase in urine excretion of porphobilinogen (PBG) that increases to more than 10 times the upper limit of normal during an attack[1]. However, urinary PBG concentrations may return to normal during remission, particularly in VP and HCP, and can be misleading in patients who have never experienced an attack. Biochemical analysis is thus only reliable in patients who have recently had clinical symptoms of an attack.

King's College Hospital is a Supra-Regional Assay Service for the porphyrias and encompasses a porphyrins laboratory, genetic service and porphyria clinic. Here we report on the first four years of providing a genetics service to patients attending the porphyria clinic at King's College Hospital for either AIP or VP from 2011 to 2015.

Method

DNA was extracted from blood or saliva using a Qiagen QIAsymphony or EZ1, respectively.

DNA sequencing was performed on the coding regions and splice junctions of the HMBS and PPOX genes using Big Dye terminators on an ABI 3130xl genetic analyser and aligned with reference sequences NM_000190.3 and NM_001122764.1, respectively. Analysis was performed using Mutation Surveyor (Softgenetics).

Results

Table 1. Summary of all variants identified and their incidence.

Gene	Mutation	Effect	Incidence
HMBS	c.33G>A	p.Ala11Ala	2
	c.34G>T	p.Glu12Stp	1
	c.269T>G	p.Val90Gly	1
	c.362G>T	p.Asp121Tyr	2
	c.422+1G>T	Delete exon 8	3
	c.517C>T	p.Arg173Gln	4
	c.601C>T	p.Arg201Trp	1
	c.604delG	p.Val202Trpfs*53	2
	c.627G>C; c.647G>C; c.649C>A	Unknown	1
	c.652-83C>T	Introduces splice site	1
	c.656C>A	p.Ala219Asp	1
	c.673C>T	p.Arg225Stp	4
	c.674G>A	p.Arg225Gln	1
	c.731_732delCT	Frameshift (stop + 5)	1
	c.739 T>C	p.Cys247Arg	1
	c.826-2A>C	Removes splice site	1
	c.826-2A>G	Intron 13 retention	1
	c.886C>T	p.Gln296Stp	1
	c.1004G>A	p.Gly335Asp	1
PPOX	c.175C>T	p.Arg59Trp	5
	c.503 G>A	p.Arg168His	1
	c.884T>C	p.Leu295Pro	1
	c.1019_1021_delGAT	Removes 340Asp	1
	c.1076delG	p.Ser359Thrfs*7	1
	c.1098+2T>G	Splice site	1

Red indicates variant not previously described in the literature.

HGVS nomenclature called against RefSeq sequence NM_000190.3 (HMBS) and NM_001122764.1 (PPOX).

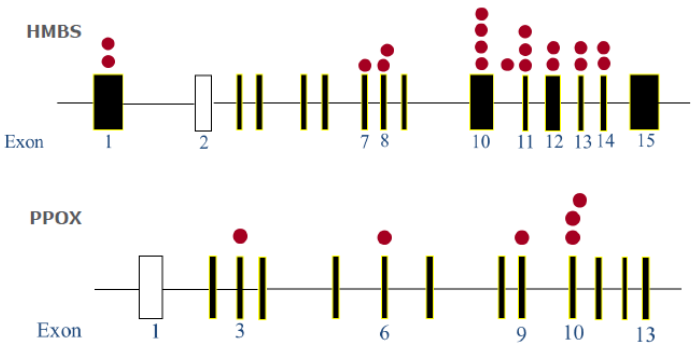

Figure 1. Schematic diagram of the HMBS (top) and PPOX (bottom) genes showing the location of the variants identified in Table 1. Non-coding exons are indicated in white, coding exons in black and variants in red.

For AIP 20 different mutations were identified in the HMBS gene, of which two had not been previously described.

For VP 6 different mutations were identified in the PPOX gene, of which two had not been previously described.

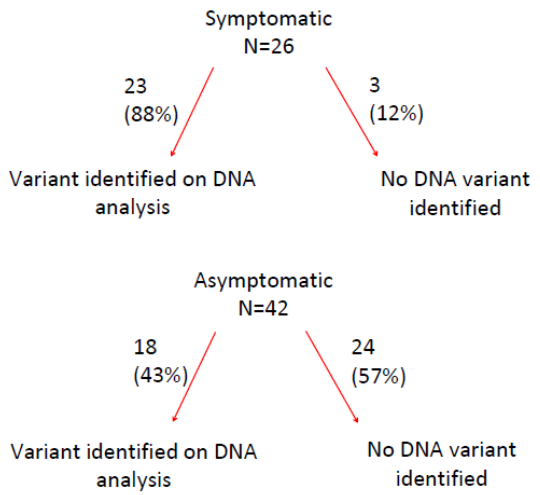

Figure 2. Breakdown of the distribution of positive DNA (variant identified) patients based on their symptoms. Symptomatic = clinical symptoms and/or positive biochemistry indicating acute porphyria. Asymptomatic = no clinical or biochemical evidence of porphyria.

Conclusions

We have established a porphyria genetics service using DNA extracted from blood or saliva. In this time we have shown genetics to be a useful tool in the diagnosis of asymptomatic patients.

We have identified four new genetic variants in the HMBS and PPOX genes which have not been previously described.

Reference: 1. Sandberg S, Elder GH. Diagnosing acute porphyrias. Clin Chem 2004; 50: 803-5

A neuro-metabolic work-up for the investigation of Seizure disorders - a one-stop approach

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Neuro-metabolic causes of Epilepsy - Why investigate adult epileptics?

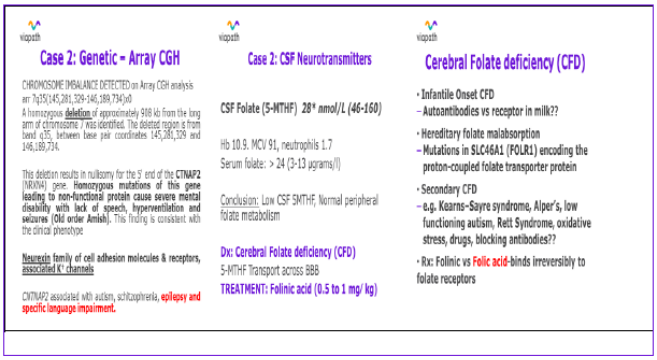
- Epilepsy in adults of childhood-onset may be due to metabolic causes, with genetic implications.
- These patients were reviewed in adult neurology clinics for management of their seizures – often associated with physical and intellectual disabilities
- Response to anti-epileptic drugs is more limited in older epileptics, resulting in poly-pharmacy to manage seizures.

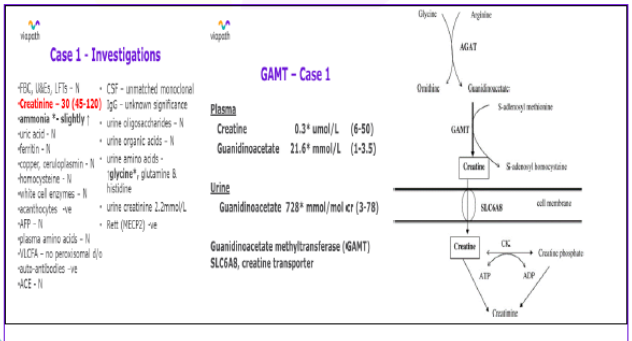
Methods – Developing a Neuro-metabolic work-up

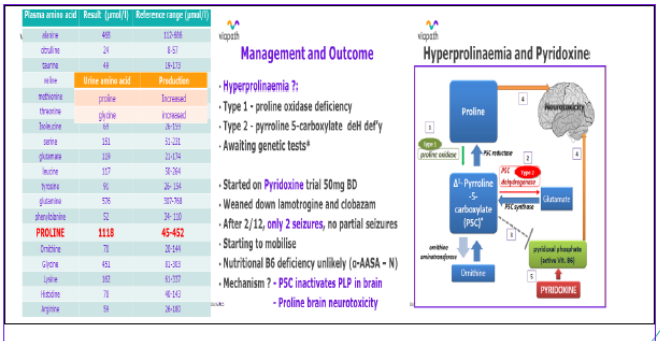
- As a tertiary referral centre for paediatric liver transplantation the diagnose of metabolic liver disease requires a co-ordinated approach
- We adapted our expertise using a multi-disciplinary investigative pathway to develop a neuro-metabolic work-up
- utilising blood, urine and CSF samples for employing recent analytical developments genetic, biochemical and enzymatic to diagnose seizure disorders
- As patient sedation is often required for lumber puncture, a one-stop approach was applied

3 Adult cases with syndromic epilepsy

- Initially normal development - Childhood onset epilepsy
- Intractable – poly-pharmacy (cost)
- Multiple hospital admissions (cost)
- Syndromic: Family history of epilepsy WITH
- Wheelchair dependence**, Speech/language delay
- Learning Difficulties**, behavioural disturbances**
- Incontinence & feeding difficulties (PEG)
- **Need for sedation







Conclusion

- Using a One-Stop approach we performed work-ups to investigate 66 patients (15 with the full protocol);
- Targeted therapy improved symptoms demonstrating the utility of re-assessing adult epileptics using a rational investigative protocol, 31 Adults/Teens with paediatric onset Seizures, 30 Paediatric, 5 Adult onset Seizures.
- Our findings to date:
- *Creatine-deficiency (GAMT) syndrome in two siblings,
- *2 SCN1A mutations (channelopathies),
- *2 Hyperprolinaemia responsive to Pyridoxine treatment
- *Cerebral Folate deficiency with a genetic defect
- *GLUT-1 disorder (SLC2A1 mutations)
- *Several significant abnormalities on array CGH

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Development of a high-resolution mass spectrometric immunoassay (MSIA) for human hepcidin

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Introduction

- Hepcidin is a 25-amino acid polypeptide (MW 2789) that is considered to be the key regulator of systemic iron homeostasis.
- Measurement of hepcidin may aid the differential diagnosis of iron deficient anaemia, or anaemia of chronic disease, and may also help guide the treatment of anaemia with iron supplementation, erythropoietin or with novel therapies.
- Truncated isoforms of hepcidin-25; hepcidin-20 (MW 2191), -22 (MW 2436), and -24 (MW 2674) have been identified but their role has not yet been defined.
- MSIA involves the immunoenrichment of an analyte on a monolithic microcolumn which is activated with an anti-protein antibody fixed in a Disposal Automated Research Tips (D.A.R.T). The analyte is then eluted, and analysed by LC-MS(MS).
- A number of methods are available for the measurement of plasma hepcidin-25; however, there are concerns over the and poor agreement between methods has been reported
- MSIA has the required selectivity and sensitivity to make it suitable for the measurement of hepcidin-25.
- The aim of this work was to investigate the viability of MSIA for the quantification of hepcidin-25, and its isoforms.

Method

- DART'S were pre-bound with anti-hepcidin-25 antibody.
- Calibration solutions containing hepcidin-25 and isoforms over the range; 1–100 µg/L were prepared in phosphate buffered saline (PBS) containing 10 g/L (v/v) bovine serum albumin.
- Sample (200 µL) was diluted with internal standard solution [hepcidin-25-$^{13}C_{18}$,$^{15}N_3$ (48 µg/L), in HBS-EP buffer, 500 µL], and captured as per **Table 1**.
- Captured analytes were eluted from the DART'S using 33 % (v/v) acetonitrile (aq) containing 0.4 % aqueous trifluoroacetic acid.
- Samples were analysed using a Thermo Scientific™ Transcend™ II system with a Thermo Scientific™ Q-Exactive™ high-resolution mass-spectrometer.
- The following step-wise gradient was used: Starting condition 15 % B, ramped to 100 % B over 5 minutes, held for 1 minute then returned to initial conditions for 3 minutes.
- LC-MS parameters are given in **Table 2**. MS detection was carried out in positive ionisation mode using heated electrospray ionisation [spray voltage 4.5 kV; temperatures: vaporiser 200 °C; capillary 320 °C; auxiliary, sheath, and sweep gases 5, 50 and 0 (arbitrary units) respectively, S-lens voltage 100 V]. The C-trap capacity was set at 3 x 10^6 charges. Maximum injection time was 250 ms.
- The peak areas for the six most abundant isotopes of the +3, +4, and +5 charge states (example total ion chromatogram in **Fig. 1**) for each analyte were summed to create an extracted ion chromatogram (**Fig. 2**, mass extraction window: ± 5 parts per million).

Table 1. *Immunocapture procedure.*

Step	Solution	Solution volume (µL)	Number of cycles	Cycle volume (µL)
1 - Wash	Water	200	20	150
2 - Capture	Sample	700	500	250
3 – Wash	PBS	200	20	150
4 – Wash	Water	200	20	150
5 - Elute	Eluent	100	100	75

Table 2. *LC-MS parameters.*

Parameter	Conditions
Column:	ACE C18 (100 x 2.1 mm); 60 °C
Eluents:	A: 0.1 % (v/v) aqueous formic acid B: 0.1% (v/v) formic acid in acetonitrile Flow-rate 0.25 mL/min
Injection volume:	100 µL
Detection:	Full-scan (resolution 140,000, *m/z* 400–1,000)

Acknowledgements
- We thank Viapath for the award of an Innovation Fund and ThermoFisher Scientific for providing instrumentation and DARTs

Figure 1. *Total ion chromatogram of hepcidin-25.*

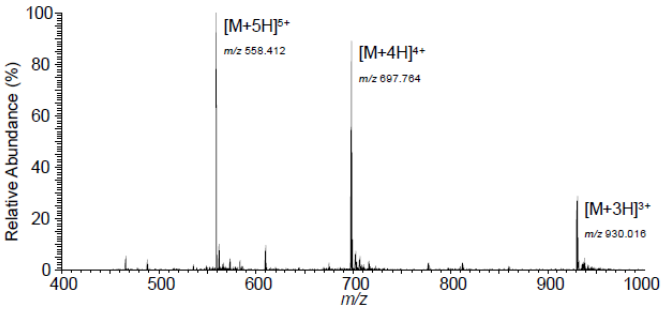


Figure 2. *Extracted ion chromatogram for (A) hepcidin-20, (B) hepcidin-22, (C) hepcidin-24, (D) hepcidin-25, and (E) hepcidin-25-$^{13}C_{18}$,$^{15}N_3$.*

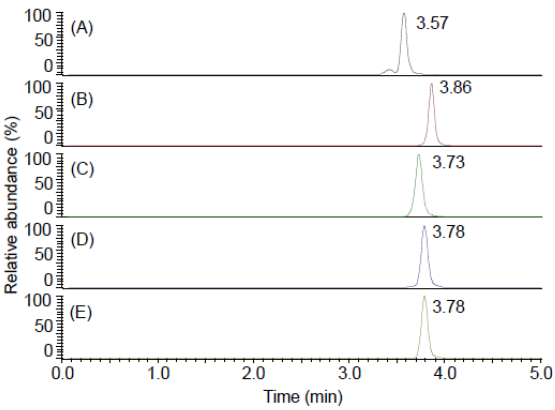
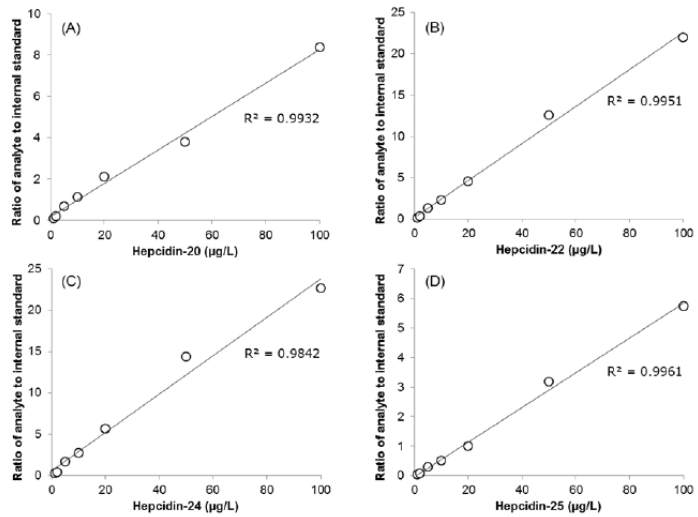


Figure 3. *Calibration curves for: (A) hepcidin-20; (B) hepcidin-22; (C) hepcidin-24; and (D) hepcidin-25.*



Results and Discussion

- All calibrations were linear over the range studied (R_2 > 0.99, **Fig. 3**), and precision (% CV, N = 3) for all analytes at 100 µg/L was < 1 %.
- Overall recovery from dH_20 (all analytes 100 µg/L) was: 56, 60, 73, 62 and 64 %, for hepcidin-20, -22, -24, -25, and the internal standard respectively.
- The proposed method is simple, easily automated, and shows the potential for MSIA in the analysis of hepcidin-25 and isoforms.
- MSIA is an ideal workflow solution for analytes which are present at low concentrations, and where interference from similar compounds is likely with conventional immunoassays.

Steroid Metabolism in relation to Fat Distribution and Insulin Resistance in Polycystic Ovarian Syndrome

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[2]Plymouth University Peninsula Schools of Medicine & Dentistry, Centre for Clinical Trials and Population Studies, Plymouth
[3]Pool Health Centre, Cornwall
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Introduction

- Polycystic ovarian syndrome (PCOS) is a heterogeneous disorder affecting up to 20% (by Rotterdam criteria) of women of reproductive age. Hyperandrogenism plays an important role in the pathophysiology of PCOS and is associated with insulin resistance and obesity.
- We assessed the effects of fat distribution and insulin resistance on steroid metabolism and evaluated the usefulness of a serum 'steroid panel' using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in PCOS.

Methods

- The cross-sectional study recruited 33 PCOS women (14 lean & 19 obese) and 30 control women (16 lean & 14 obese) matched for age and body mass index (BMI) (***Table 1***).
- Fat distribution was assessed by waist circumstance and BMI. Insulin resistance was measured using Homeostatic Model Assessment (HOMA-IR).
- All underwent an oral glucose tolerance test (OGTT, 75g) after an overnight fast. Blood samples were collected at baseline, 15, 30, 60, 90 and 120 minutes.
- Serum 'steroid panel' (androstenedione, testosterone, 17OH-pregnenolone, 17OH-progesterone, dehydroepiandrosterone sulphate, corticosterone, cortisol & cortisone), was measured by an in-house method using LC-MS/MS (TSQ Vantage, ThermoFisher Scientific) (***Figure 1***).

Results

- The insulin resistance was similar between the control and PCOS groups (***Table 1***). However, when the groups were further divided into lean and obese, insulin resistance was higher in obese PCOS and controls compared to the lean PCOS and controls.
- The baseline measurements of serum steroid panel showed increase in testosterone (p=0.02), 17OH-pregnenolone (p=0.004) and corticosterone (p=0.02) in the PCOS group vs. control group (***Table 2***). The 3 steroids did not show difference when compared between lean control/PCOS and obese control/PCOS groups, even though steroids were relatively higher in lean and obese PCOS.
- No correlation was found between the steroids measured and BMI or HOMA:IR, suggesting that changes found in steroid metabolism are likely to be due to PCOS.
- The delta response (difference between baseline and maximal response), to OGTT, was attenuated for androstenedione (p=0.005) and cortisol (p=0.04) in the PCOS group vs. control group.

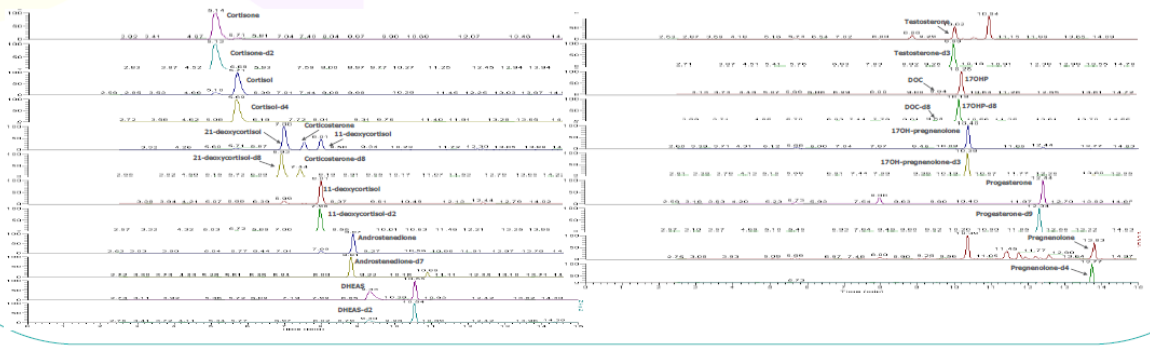
Table 1. Data is presented as medians (inter-quartile ranges). NS = no significant difference

	Control (n=30)	PCOS (n=33)	P value
Lean	**16**	**14**	**NS**
Obese	**14**	**19**	**NS**
Age	**35 (32-39)**	**32 (27-37)**	**NS**
BMI	**26 (24-31)**	**27 (23-29)**	**NS**
Waist/Hip ratio	**0.6 (0.5-0.8)**	**0.7 (0.6-1.0)**	**NS**
HOMA:IR	**0.8 (0.8-0.9)**	**0.9 (0.8-0.9)**	**NS**

Table 2. Baseline measurements of serum steroids. Data is presented as medians (inter-quartile ranges). NS = no significant difference.

Serum steroids (nmol/L)	Control	PCOS	P value*
Androstenedione	2.5 (2.1-3.2)	3.3 (2.3-5.0)	0.08
Testosterone	0.9 (0.7-1.1)	1.1 (0.9-1.4)	0.02
17OH pregnenolone	2.9 (2.0-5.9)	8.5 (4.0-9.9)	0.004
17OH progesterone	3.0 (1.8-4.0)	2.3 (1.7-3.8)	NS
Corticosterone	13 (9-20)	25 (15-41)	0.02
DHEAS (µmol/L)	3.8 (2.4-6.6)	5.3 (2.8-6.9)	NS
Cortisol	268 (93-432)	368 (222-699)	NS
Cortisone	53 (45-66)	56 (51-64)	NS
SHBG	56 (36-78)	52 (31-88)	NS
FAI	1.6 (0.8-2.1)	1.8 (1.0-3.2)	NS

Figure 1: Serum steroid panel. LC chromatograms showing resolution of steroids and their corresponding deuterated standards



Conclusion

- The changes in steroid metabolism in our cohort were more likely due to PCOS rather than fat distribution/insulin resistance.
- Higher 17OH-pregnenolone in PCOS has not been previously reported.
- It remains to be elucidated if the altered steroid metabolism is a contributing factor to the pathogenesis or a consequence of PCOS.
- A serum 'steroid panel' provides advantages over single steroid measurements in excluding major causes of androgen excess.

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Automation of Technozym ADAMTS-13 assays on the Dynex DS2™

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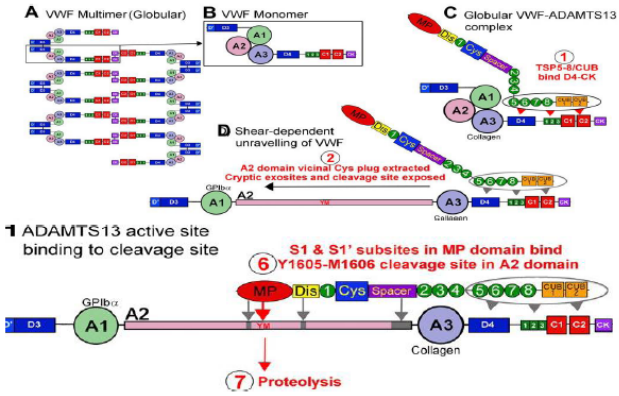
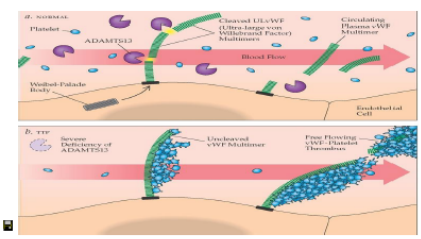
Introduction

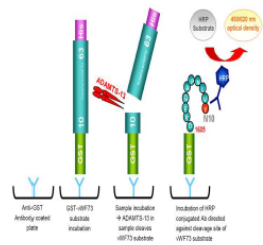
ADAMTS-13 is a metalloprotease that cleaves large von Willebrand factor (VWF) multimers into smaller, less reactive multimers. Congenital or acquired deficiency of ADAMTS-13 leads to the potentially lethal syndrome of thrombotic thrombocytopenic purpura (TTP). Acquired TTP is usually due to autoantibody development, or can arise from massive endothelial activation releasing large amounts of ultra-large VWF multimers sufficient to overwhelm degradation capacity of ADAMTS-13.

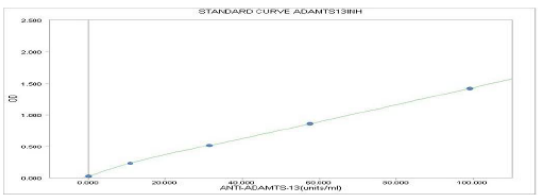


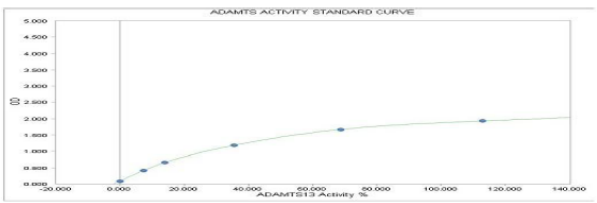
Method

The Technozym ADAMTS-13 activity and inhibitor assays (pathway diagnostics) are chromogenic assays employed to detect deficiency of ADAMTS-13 and the presence of an inhibitor to distinguish between congenital and acquired TTP. The activity assay is an indirect functional ELISA whereby a recombinant VWF fragment (GST-VWF73-His) is immobilised onto the plate. When the plasma is added, cleavage of the immobilised fragment occurs at the ADAMTS-13 cleavage site. The residual cleaved VWF fragment is measured so ADAMTS-13 activity is therefore inversely proportional to the residual substrate concentration. The inhibitor assay is a conventional ELISA assay. Protocols were written and optimised for both assays on the Dynex DS2 ELISA analyser (Werfen Ltd) in line with the manufacturer's assay performance instructions and using our departmental biovalidation procedures to assess precision , accuracy and reference ranges.









Results; ADAMTS-13 activity reference range derived from 2.5th -97.5th percentiles of results from 55 normal donors was 66.4-107.9%. In view of similarities between the manufacturer's cut-offs for ADAMTS-13 inhibitor (n=193) and locally derived values (n=39), they were merged to give the following cut-offs for diagnostic testing: Negative:<10U/mL, Boderline:10-15U/Ml, Positive:>15U/Ml. The ADAMTS-13 activity and inhibitor assay are enrolled on the ECAT EQA scheme. Results from the 2015.1 survey are shown below.

Assay	ECAT Assigned Value	Our result	Z-Score
ADAMTS-13 Activity	32	26	-1.07
	6	6	-0.21
ADAMTS-13 Inhibitor	6.7	4.71	-1.09
	14.2	12.0	-0.66

ACCURACY			
ADAMTS-13	Target (U/mL)	% from target	Result
Activity	93.9	4.6	89.6 (n=4)
Inhibitor	99.0	6.87	93.2 (=3)

ADAMTS-13 intra assay precision	Normal control target (U/mL)	CV%	Low control target (U/mL)	CV%
Activity	79 (n=10)	0.02	ND	
Inhibitor	49 (n=10)	2.47	0.49 (n=10)	0.15

ADAMTS-13 inter assay precision	Normal control target (U/mL)	CV%	Low control target (U/mL)	CV%
Activity	74.9 (n=4)	4.0	21.7 (n=4)	7.0
Inhibitor	66.1 (n=3)	0.08	7.0 (n=3)	0.03

Conclusion; The automation of the Technozym ADAMTS-13 activity assays is providing a precise, rapid reporting procedure and can be urgently performed on new or follow up patients. If the ADAMTS-13 activity is <5%, we are able to perform the ADAMTS-13 inhibitor assay immediately which aids guidance to the clinical staff on treatment and prognosis options. As no international standard, quality control material or officially recognized units exist a robust validation procedure and participation in an EQA scheme is recommended. Our returned results demonstrate that we are performing adequately for these assays. The automation of these assays on the Dynex DS2 has demonstrated the protocol writing flexibility of the analyser software.

Acknowledgements: We would like to thank Pathway Diagnostics and Werfen for their helpful scientific and technical advice during the automation project.

References; (1) Sadler JE.A new name in thrombosis ADAMTS-13. proc.Nat.Acad Sc USA 2002 11552-11554, (2) Peyvandi F, Palla R, Lotta LA, Mackie I, Scully MA, Machin SJ. ADAMTS-13 assays in thrombotic thrombocytopenic purpura. J Thromb Haemost. 2010 Apr; 8(4): 631-40, (3) Diagrams taken from Google images website.

Automation of Anticardiolipin Antibody Assays on a Dynex DS2 Analyser

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Introduction

Anticardiolipin antibodies (aCL) are one of the criteria antiphospholipid antibodies (aPL) for diagnosis of antiphospholipid syndrome (APS). The other criteria for aPL are lupus anticoagulant (LA) and anti-B2-glycoprotein I (anti-B2-GPI). People with APS have risk of developing thrombotic disorders, particularly venous and arterial thrombosis. There is also an increased risk of having a miscarriage amongst pregnant women with APS. It is estimated that APS is responsible for one in every six cases of deep vein thrombosis (DVT), strokes and heart attacks in people under 50 and one in every six cases of recurrent (three or more) miscarriages [1]. Enzyme-linked immunosorbent assay (ELISA) are commonly used for the semi-quantitative detection of aCL in human serum. The presence of cardiolipin antibodies can be used in conjunction with clinical findings and other laboratory tests to aid assessing the risk of thrombosis in individuals with APS, Systemic Lupus Erythematosus (SLE) or lupus-like disorders. In this study, we evaluated the performance of Inova Diagnostics Quanta Lite® IgG and IgM ELISA reagents (Werfen Group, California, USA) on a Dynex DS2 automated ELISA analyser (Werfen Group) according to manufacturer's instructions.

Method

All evaluations were performed on the Dynex DS2 (programmable) automated ELISA analyser (Werfen Group). The assay consisted of use of the reagents: Inova Diagnostics Quanta Lite® IgG and IgM ELISA reagents (Werfen Group, California, USA). The presence or absence of aCL was determined by comparison of the sample optical density with that of a 6 point calibration curve. An absorbance (OD) of 450nm, and reference wavelength of 620nm, was used. The clinical performance of the assay was investigated by determining the following: intra-precision assay (n=12) and the coefficients of variation (CV) for inter-precision assay (n=6), using ACA IgG and IgM controls (containing preservative and human serum antibodies to cardiolipin); and lower limit of quantification. The cut-off for the assays was determined by analysis of 66 normal donor sera samples (which was kept frozen at -80°C). A comparison study was also performed by analysis of 30 patient serum samples with different reagents (Aeskulisa, Wendelsheim, Germany) and analyser (Grifols UK Triturus, Cambridge, United Kingdom).

Results

The standard curve for the IgG assay was linear throughout the standard range (*Figure 1a*) whilst the IgM assay exhibited plateauing at the higher standard (*Figure 1b*), common to many ELISA assays. The intra precision assay on control samples of IgG and IgM were 3.2% and 3.5% respectively. The CV for the inter precision IgG and IgM aCL were 8.2% and 6.6% respectively. *Table 1* shows the summary of the precision run data and reproducibility of the assays. The lower limit of quantification was determined to be 2.7GPL (IgG) and 2.9MPL (IgM). Cut-offs were derived (n=66) using 95th-99th percentile for low positives in relation to obstetric APS, moderate positives as between 99% - 80G/MPL, and strong positive, >80G/MPL. The 95th-99th percentile for IgG and IgM were 10-15G/MPL. *Table 2* summarises the in-house therapeutic ranges generated. The comparison study with different reagent and analyser had shown good agreement, with values ranging from normal to strong positive. In view of improved linearity of standard curves on the Dynex DS2 at higher levels where Triturus standard curves begin to plateau, direct comparison of patient data with higher values was not possible. However, in terms of results being 'normal, slightly, moderately or markedly elevated' results were diagnostically equivalent.

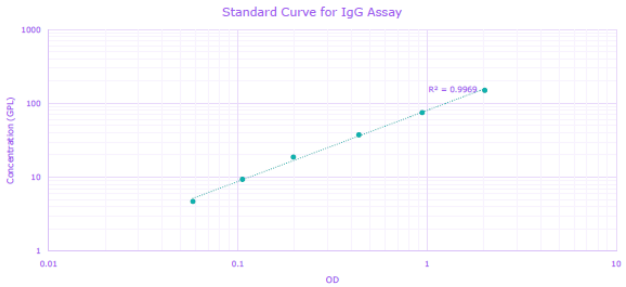

Figure 1a – Standard curve for aCL IgG assay

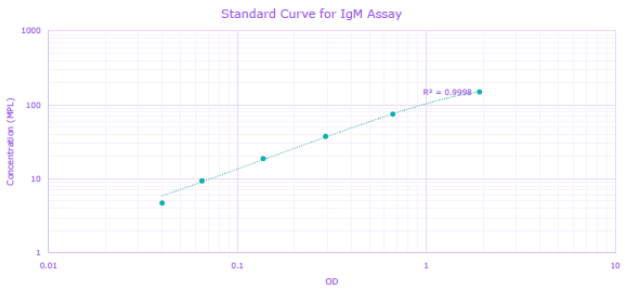

Figure 1b – Standard curve for aCL IgM assay

	aCL IgG	aCL IgM
Intra Assay (n = 12)	Range for positive GPL is 35 - 75GPL CV = 3.2% Mean = 47.54 SD = 1.51	Range for positive MPL is 35 – 75MPL CV = 3.5% Mean = 48.85 SD = 1.70
Inter Assay (n = 6)	Range for positive GPL is 35 - 75GPL CV = 8.2% Mean = 45.39 SD = 3.73	Range for positive MPL is 35 – 75MPL CV = 6.6% Mean = 47.46 SD = 3.13

Table 1 – Summary of precision studies for aCL IgG and IgM assay

IgG & IgM aCL	Range
Cut-Off	10G/MPL
Low Positive	10 – 15G/MPL
Moderate Positive	>15 - <80G/MPL
High Positive	>80G/MPL

Table 2 – Description of reference range

Conclusion

From our data, we confirmed the pairing of Inova aCL reagents on Dynex DS2 analyser generated robust assay suitable for detecting and quantifying aCL according to current guidelines. It is a simple and reliable assay which has vastly improved turn around times for testing. It provides ideal patient monitoring for suspected cases of APS, SLE or other thrombotic disorders, within the diagnostic laboratory.

Reference

[1] http://www.nhs.uk/Conditions/hughes-syndrome/Pages/Introduction.aspx (2013), last accessed on 16th August 2015.

Measurement of Rivaroxaban (Xarelto ®) in plasma using Sysmex CS2100i.

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Abstract

Patients requiring anticoagulant therapy are increasingly prescribed direct oral anticoagulants (DOACs). Advantages include the ability to prescribe a fixed oral dose with little requirement for close monitoring and dose adjustment. There are certain clinical situations where circulating concentration values are required including: pre-surgery; bleeding; suspected drug overdose; impaired renal function and presence of interfering drugs. The availability of an accurate and sensitive assay for DOACs is critical.

Presently, the direct Xa inhibitor rivaroxaban is increasingly prescribed within Guys and St. Thomas' Foundation Trust. To facilitate clinical need, the Stago STA-Liquid anti-Xa kit in conjunction with Stago calibrators and controls were validated to measure plasma rivaroxaban. The protocol was modified for use on the Sysmex CS2100i.

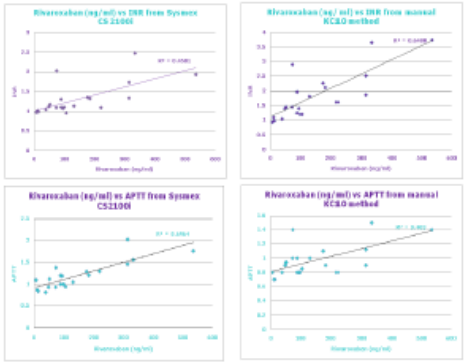
The calibration curve maintained linearity to approx. 17ng/ml prompting the introduction of dilutions between 100ng/ml and 20ng/ml to improve accuracy. Validation experiments generated inter-assay precision of 4%(n=5) and intra-assay precision of 5.7%(n=9). The assay was further shown to maintain stability for at least a week.

Samples from patients (n=21) known to be taking rivaroxaban were assayed using the optimised protocol for the liquid anti-Xa assay and results compared to prothrombin times (PT) generated with rabbit brain derived thromboplastin using the manual KC10 method and recombinant human thromboplastin (Innovin) using the automated Sysmex analyser. A greater correlation was achieved with the manual KC10 method (0.9 vs 0.6), reflecting the increased sensitivity of the rabbit brain derived thromboplastin versus human recombinant thromboplastin to rivaroxaban.

The assay has been incorporated into the laboratory's repertoire and is in continual use.

Fig 1: Comparison of the mode of action of common anticoagulants

Fig 2: Plasma rivaroxaban concentrations compared with results from standard coagulation tests.



Materials and Method

The STA-Liquid anti Xa kit for the calorimetric assay of heparins, fondaparinux and rivaroxaban was assessed in conjunction with the STA rivaroxaban calibrators and controls (Stago). The manufacturers assay method was adapted for use on the Sysmex CS2100i. Initial curves generated with the neat standards did not exhibit linearity prompting the inclusion of dilutions into the protocol. Optimum linearity was observed between 17ng/ml and 200ng/ml, however the curve was extended to facilitate measurement up to 500ng/ml as a primary objective of the test was to identify rivaroxaban overdose. A final curve employing 6 rivaroxaban concentrations was demonstrated to have inter (n=5) and intra (n=9) precision of 4% and 5.7 % respectively.

A retrospective analysis of patient samples was conducted. The rivaroxaban measured was compared with results from coagulation tests performed using the CS2100i and the manual KC10. The CS2100i PT employed Innovin® recombinant thromboplastin reagent and Actin FS® reagent (contact activator ellagic acid) for activated partial thromboplastin time (APTT) analysis. The manual KC10 utililsed rabbit brain derived thromboplastin (Thromboplastin LI Manchester reagent) for PT and TriniClot FS reagent (contact activator silica) for APTT determination.

Table 1: Pearson's r correlation coefficient for rivaroxaban and:

Rivaroxaban concentration	All (n=21)	<100 ng/ml (n=12)	>100 ng/ml (n=9)
INR (Sysmex CS2100i)	0.63	0.35	0.71
APTT (Sysmex CS2100i)	0.83	0.47	0.8
INR manual KC10 method	0.89	0.46	0.82
APTT manual KC10 method	0.63	0.38	0.69

Discussion

This study aimed to establish a reliable and reproducible anti Xa assay for the determination of circulating rivaroxaban concentration. Protocol modification permitted the use of the Sysmex CS2100i platform, currently used routinely for clotting and chromogenic assays within the laboratory. The assay will in due course be adapted for measuring other DOACs.

Results obtained from patients prescribed rivaroxaban were compared to routine coagulation tests. INR's obtained using recombinant and rabbit brain derived thromboplastins gave correlations (Pearson's r) of 0.68 and 0.81 respectively (see Table 1). Correlation values obtained using the PT in seconds were not markedly different (0.67 and 0.83) in contrast to the findings of previous publications [1]. The data support the evidence from previous work[1] and the SSC recommendation[2] that use of the PT for rivaroxaban monitoring is thromboplastin type dependant.

Plasma rivaroxaban concentrations and APTT showed a better correlation with the automated assay than the manual (0.83 vs 0.63 respectively).

Further analysis of the PT and APTT data relative to the rivaroxaban concentration revealed that the best correlation only occurs where plasma rivaroxaban is greater than 100ng/ml (Table 1).

Circulating plasma levels of DOACs, including rivaroxaban ,are not routinely monitored and are often required urgently. The discrepancies between PT, APTT and rivaroxaban concentrations, particularly at low doses, established the anti-Xa based assay as the most appropriate test where there is a clinical need to assess the plasma rivaroxaban status of a patient.

References: (1) Samama M.M. *et al.*, (2013) Thromb J. 11: 11-17 (2)Baglin T. *et. al.*, (2013) J Thromb Haemost 11: 756-760

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