Issue 5: April 2017

pathology@viapath

Message From The Editor

Welcome to pathology@viapath which I hope gives you an insight into some of the pioneering pathology work taking place within Viapath and has articles of particular interest to you.

Like me, did you eat too much chocolate over the Easter period? Thinking of dieting? If so, you may want to read "Detectives at Work", particularly if you are thinking of purchasing diet pills, because one of the compounds is a laxative that has been withdrawn from use due to concerns about carcinogenity!

This winter, I don't know if you had one of nasty colds that were doing the rounds? The good news is that Viapath has made significant advances in the testing for respiratory tract infections and, with a quicker and more accurate diagnosis, these are now reaping benefits for both patients and hospitals.

If you would like to know how the lives of babies diagnosed with phenylketonuria through the newborn screening programme are transformed, then the report on "Rare Disease Day" will fascinate you.

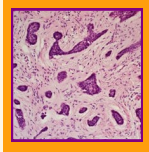
Inside This Issue

Detectives At Work: Analysing An Unknown Substance

- Analysis of an unknown substance can be extremely useful in clinical diagnosis
- Substances ranging from powders and pills to herbal material, cigarette stubs and liquids can be analysed using high-resolution accurate mass spectrometry
- Viapath's Toxicology Laboratory determined the components of a 'diet pill' bought online

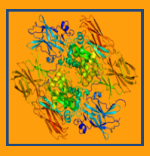
Viapath Celebrates Rare Disease Day

- The main objective of Rare Disease Day is to raise public awareness of rare diseases
- Viapath's Rare Disease Day event, focused on phenylketonuria (PKU), a disease that affects 1 in 10,000 babies a year and is diagnosed through the Newborn Bloodspot Screening programme



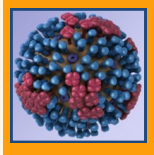
An Evaluation Of An Automated Platform For The Histological Assessments Of Non-Melanoma Skin Cancer

- Diagnosis of skin cancer, and in particular non–melanoma skin cancer, has been steadily increasing.
- Current methods for frozen tissue sectioning are inadequate and imprecise
- Dr Guy Orchard gives an assessment of two new pieces of equipment on offer that are proposed to improve efficiency and accuracy in histology



The Importance Of Using A Acceptable Linear Calibration Curve For Coagulation Factor Assays

- Haemostasis is the balance of maintaining blood in a fluid state within blood vessels and limiting blood loss after trauma
- The imbalance of coagulation factors can have implications in disease states such as venous thromboembolism, atherothrombosis or cardioembolic stroke
- One-stage factor assays are used to determine whether a patient has sufficient coagulation activity to maintain haemostasis



Breathing New Life Into The Diagnosis Of Respiratory Infections

- Respiratory tract infections are a major cause of morbidity and mortality, particularly in the very young, elderly and those with underlying health conditions
- Viapath has made significant advances in the testing of respiratory tract infections
- This has resulted in better patient outcomes and improved hospital practices

Under The Microscope: Dermatopathology

- Insights into Viapath's Dermatopathology Laboratory

Detectives At Work: Analysing A Substance Of Unknown Origin

Analysis of an unknown substance can be extremely useful in certain scenarios. For example, using toxicological analyses to identify substances (e.g. tablets, powders, liquids) found on a patient may aid clinicians in cases of poisoning, particularly if the patient is unconscious. Substance identification may also be of use when an individual is taking non-prescribed medications (e.g. dietary supplements or herbal remedies) as the analysis may aid the clinician in discerning what has caused a change in health.

In recent years, the market for dietary supplements has grown and many products are easily available on the internet. These products lack any formal regulation or quality control, and despite being advertised as 'natural' and 'safe', some may in fact contain pharmaceutical drugs or ingredients that are known to either interact with other prescription drugs or are toxic. In terms of illicit drugs, a parallel can be drawn with novel psychoactive substances (often marketed as 'legal highs') where, due to the rapidly changing production of these compounds, users may be unaware of what they are actually taking.

What could a "Diet Pill" contain?

Viapath's Toxicology laboratory was approached by the producer of a new 10-part TV series called "*Don't Tell the Doctor*". The programme tackles some of the dangers of self-diagnosing, buying medication online and other medical issues affecting young people. A group of four young qualified doctors take appeals from young people in the UK who have various conditions and illnesses. Each episode helps four people find the right treatment after having wrongly self-diagnosed themselves. One of the patients had bought "diet pills" from uncertified sources; the doctor wanted to identify what was actually present in these pills and to determine whether this might account for their clinical symptoms.

A single green and white capsule was submitted to Viapath's laboratory for analysis (Figure 1). Using high-resolution accurate mass spectrometry, two compounds were identified in the capsule; sibutramine and phenolphthalein (Figure 2). Sibutramine is a serotonin reuptake inhibitor (SNRI), which has been used in the treatment of obesity. However, many countries, including the UK, have now withdrawn the drug from use due to concerns over potential adverse cardiovascular side effects (e.g. hypertension, tachycardia, myocardial infarction). Phenolphthalein has been used as a laxative, but the drug has since been withdrawn due to concerns over carcinogenicity. Both sibutramine and phenolphthalein have been reported as commonly detected components of numerous different weight-loss tablets bought over the internet. The full story was shown on 5Star on 6th April.

How can Viapath help identify unknown substances?

An "unknown substance screen" is offered by Viapath's Toxicology Department, based at King's College Hospital. Substances ranging from powders and pills to herbal material, cigarette stubs and liquids can be analysed. A urine sample from the patient may also be sent so that any compound identified in the substance can be looked for in the urine sample as well. It is best to speak directly to the laboratory before sending substances, so that we can give advice specific to the individual case.

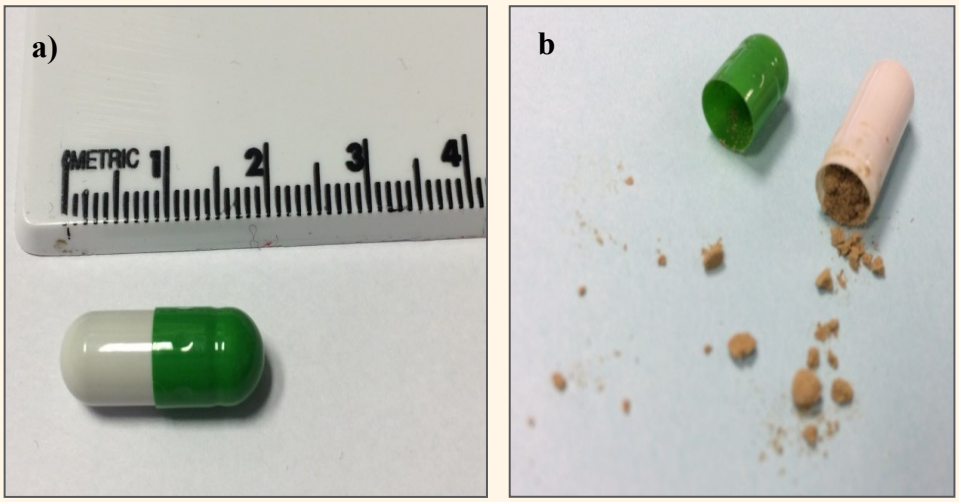


Figure 1 - Physical appearance of a) the capsule and b) the contents within the capsule

Due to the complex nature of unknown substance screening, turnaround times vary, usually taking between 5-10 working days. In some cases, identification may not be possible.

Liquid chromatography coupled to high-resolution accurate mass spectrometry is used for unknown substance screening. A solution of the unknown substance is analysed and from this the accurate mass spectrometry is used for unknown substance screening. Computer software is used to ascertain a theoretical chemical structure, which can be searched for in databases to try and identify a match. Numerous confirmation criteria are used to identify a substance, including accurate parent mass, fragment ions, retention time and isotope pattern. Where possible, any analyte identified in a substance is confirmed through analysis of a certified reference standard.

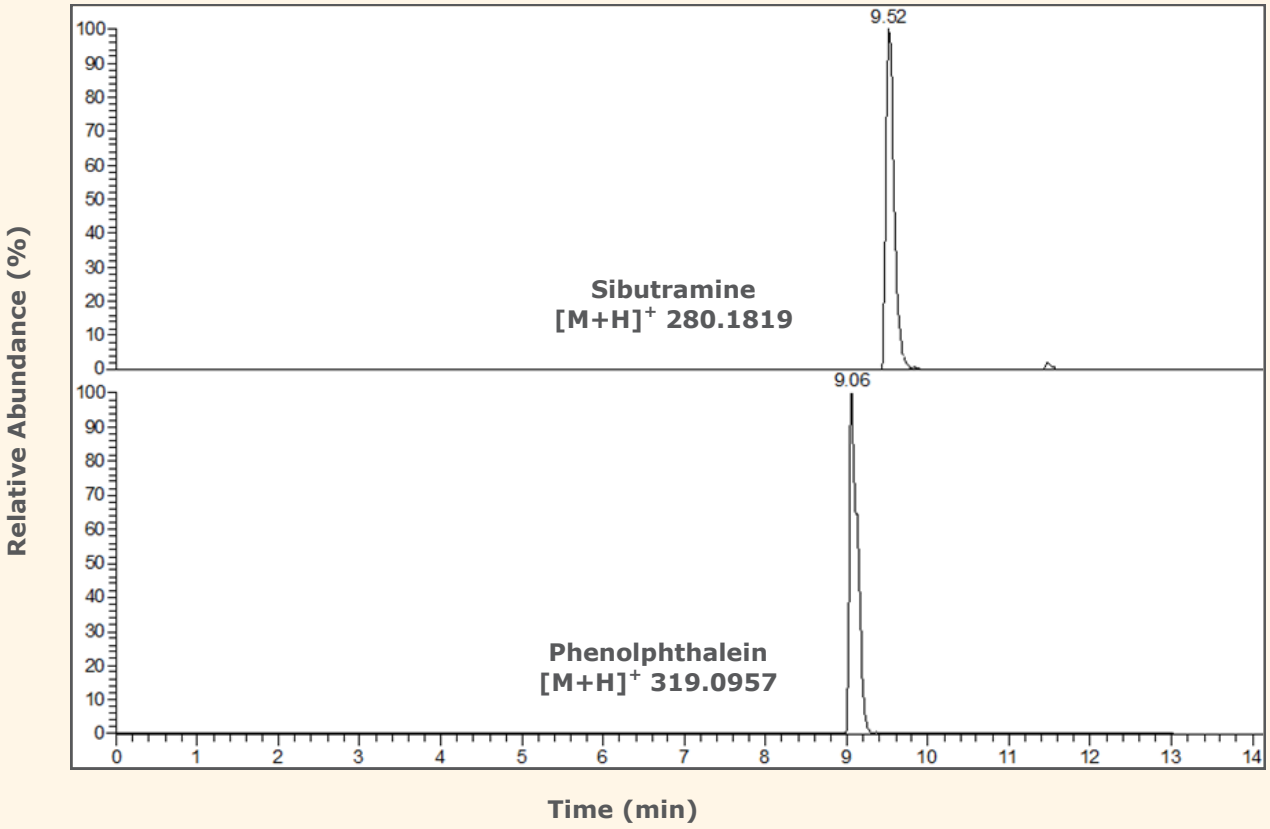


Figure 2 - Extracted ion chromatograms to show sibutramine and phenolphthalein detected in the capsule

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Relevant Publications

Viapath Urine Drugs of Abuse Screening – Information for Service Users

(http://www.viapath.co.uk/our-tests/unknown-substance-identification)

Belsey SL and Karch SB (2015) Substance Misuse: Herbal Medicine, In Encyclopaedia of Forensic and Legal Medicine (Second Edition), edited by Jason Payne-James and Roger W. Byard, Elsevier, Oxford, Pages 377-387, ISBN 9780128000557.

An Evaluation Of A Fully Automated Platform For The Histological Assessments Of Non-Melanoma Skin Cancer

Dr Guy Orchard gives an analysis of an innovative approach to improve efficiency and accuracy of frozen tissue sectioning

Skin cancer and in particular, non–melanoma skin cancer (NMSC), has been steadily increasing. The basal cell carcinoma (BCC) accounts for >80% of that recorded within the UK. These tumours can occur anywhere on the body, but are more commonly seen in sun exposed sites, such as the face, head, neck and ears. It is also the case that BCC's can occur at the sites of former burned tissue, scars or ulcers that have damaged the integrity of the skin.

How have the demographics of NMSC changed over time?

As a junior biomedical scientist, I recall seeing histology request forms for BCC's, mostly on behalf of patients in their 60's or over. As time has gone by, we have seen the incidence of NMSC rise. I now manage a large Mohs laboratory, one of the largest of its type within the UK and I see BCC's in patients in their early 30's and 40's. A large majority of these younger patients, in spite of our understanding of the effects of sun damage on skin, are strict 'sun worshippers', so have increased levels of exposure to sunlight and more specifically the ultraviolet wavelengths UVA and UVB.

Mohs practice within the UK has been expanding steadily over the last decade. Classically, Mohs procedures have the benefit of minimal excision margin clearance and therefore improved preservation of the surrounding uninvolved tissue. In addition, complete tumour clearance following Mohs procedures is assured with accuracy values of over 95%, which remains unmatched by any other patient management approach in skin cancer treatment.

Current equipment for frozen section analysis

In conventional cryostats the method is fraught with inaccuracies including tissue distortion and, most significantly, uneven embedding at the chuck face (the place where the tissue meets the cryostat knife for sectioning) which leads to inadequate and inconsistent tissue orientation problems. These issues become more apparent the smaller and more fragile the tissues are.

Figure 1 - Key features of the PrestoChill Cryoembedding System

The increased pressure to meet TATs has led to the popularity of liquid nitrogen because it has the benefit of reducing the freezing time and the number of freezing artefacts. However, it is often less precise in terms of accuracy of tissue orientation.

A wide range of devices currently produced have concentrated on the flattening of tissue at the surface of the chuck prior to tissue sectioning in an attempt to improve accuracy. However, simply applying a flattening force across the tissue surface during the freezing steps is not always the answer, as tissue composition often affects the ability to lay such tissue down flat without curling or folding. The new generation of embedding devices will need to combine benefits of rapid freezing with concepts of tissue flattening and good orientation. Orientation of tissue will also depend on good visualisation as well as manipulation of the tissue during the freezing process. The issue is a complex one!

A total of 250 blocks were embedded on the PrestoChill and stained on the Presto benchtop processor/stainer. All the tissues selected were assessed in parallel with conventionally frozen tissue to compare and contrast the quality, speed, efficiency and accuracy of both the devices. Figure 1 illustrates the key components and features of the device.

How can a modern piece of equipment manage these variables and perhaps improve efficiency and speed?

In June 2016, Menarini approached Viapath's laboratories at St. John's Institute of Dermatology to undertake an evaluation of some new pieces of equipment; the PrestoChill Cryoembedding System and the Presto automated processor/stainer. These two devices have efficiencies of speed over conventional manual methods of frozen tissue section production and staining. Manufactured for use in all forms of frozen section work, both pieces of equipment are designed to be used in a combined unified approach to the histological assessment of frozen section production and subsequent Hematoxylin and Eosin (HE) staining. This includes their application in a host of specialist histological procedures that, due to their complexities, require a more precise tissue analysis.

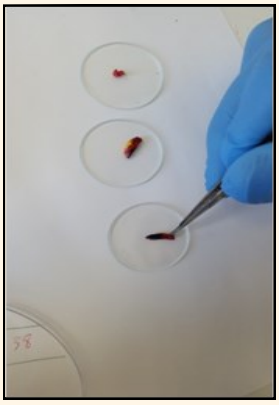

Figure 2 - Tissue on the embedding platform (either spatula or glass disc) to ensure opitimal orientation prior to loading into the PrestoChill Cryoembedding System device

The use of an optimising cryoembedding compound called MCC, in conjunction with PrestoChill paper to rest the tissues on before freezing, are new developments (figure 2). The speed of embedding was confirmed to be no more than 60 seconds with all the tissues tested. There are also four embedding wells (figure 3) which increases the speed at which multiple tissue slices can be assessed. The PrestoChill device has shown that complex tissues can be embedded correctly and effectively. The options of wells with deeper depths can be used and easily inserted to replace the normal wells provided with the machine, which is beneficial with embedding denser or thicker tissue pieces. Having embedded and sectioned the tissues, the use of the Presto processor/stainer device presents more features (figure 4).

The device can handle 6 slides (figure 5) at a time and the fixation, clearing and dehydration steps were consistently achieved within 1 minute on all the tissue sections stained. The HE staining itself is efficient and the elimination of lengthy washing steps enables the speed of staining to be, on average, less than 3 minutes. Programme adjustments can be easily made to lengthen staining steps to account for operator choice, a surgeon's preferences for staining intensity and contrast, and thicker tissue sections.

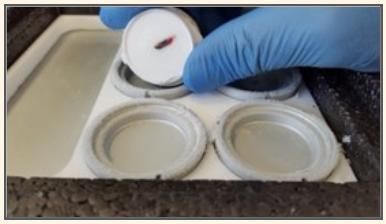

Figure 3 - The freezing embedding chambers/wells of the PrestoChill Cryoembedding System. Two wells are 25mm in diameter and two are 35mm in diameter.

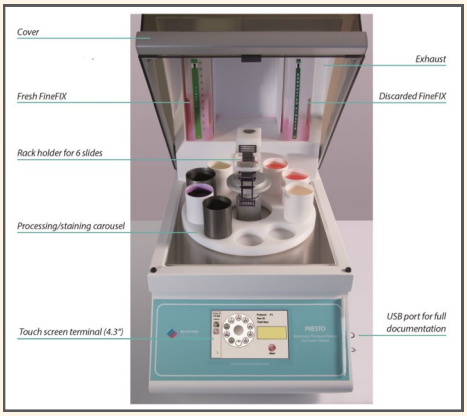

Figure 4 - Key features of the Presto automated processor/ stainer

Figure 5 - Presto automated processor/ stainer slide rack showing a six-slide capacity.

The future of frozen tissue sectioning

These complementary devices are very exciting developments in the field of fresh frozen section preparation and staining. They represent a comprehensive approach to tackling a whole gambit of technical challenges faced in producing good quality and well-stained frozen sections. Within the UK, such devices are likely to gain exposure within the field of Mohs procedures, mainly because of the evident improvements in speed and efficiency. The clean and compact nature of both devices also embraces benefits in the LEAN environment of laboratory design. The benefits of an almost fully automated process are improved standardisation of practice and, ultimately, improved overall quality of performance.

History tells us a lot, a brief glance back over the last 20-30 years within the field of tissue and cellular science has shown us how automated platforms have been embraced and have standardised practices generally for the better. Perhaps it is now time to review this in the light of our frozen section work too.

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Breathing New Life Into The Diagnosis Of Respiratory Infections

Since December 2015 there have been significant advances in the way Viapath's Infection Sciences Laboratory at St. Thomas' Hospital tests for respiratory tract infections. The development work that enabled these advances was partially funded by a grant from Guy's and St. Thomas' Charity.

What is the burden of respiratory tract infections?

Respiratory tract infections are an important cause of morbidity and mortality, particularly affecting the very young, the elderly and those with underlying health conditions. These infections not only place pressure on the NHS but also contribute to the loss of many working days and the knock-on financial and social impact on society as a whole.

"This is an excellent example of service development significantly benefitting patients." Dr. Siobhan O'Shea, Lead Clinical Scientist

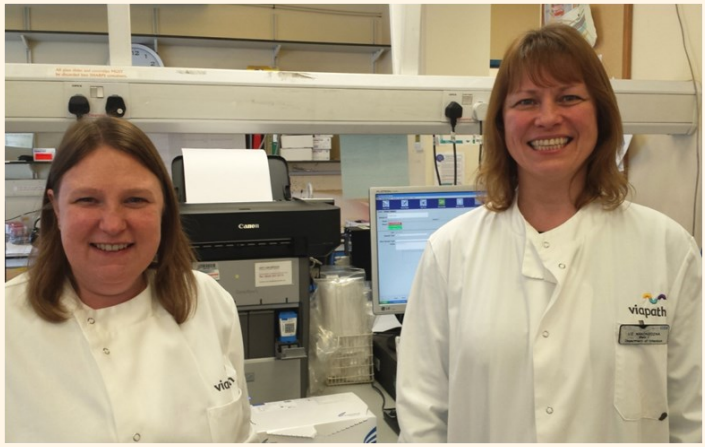
During the winter months hospital admissions due to influenza place a significant pressure on availability of hospital beds and this has been highly publicised in the mainstream media in recent years. Public Health England figures for winter 2015-16 state that there were 2462 Intensive Care Unit (ICU) admissions due to influenza infections, resulting in 209 deaths[1]. Timely diagnosis of influenza is important both for infection control and for implementing treatment and prophylaxis with antiviral agents.

Community acquired pneumonia (CAP) also peaks in the winter, although it does occur all year round. The incidence of CAP is approximately 5-11 cases per 100 000 population, with about one fifth needing hospital admission. About 5-10% of these patients need intensive care and half of those will die. In approximately half of all pneumonia cases, the causative pathogen is not identified due to limitations of some diagnostic methods. For example, some pathogens do not grow well or at all on standard microbiological media, serological diagnosis is too slow and can be inaccurate, and historically molecular tests had slow turnaround times due to referral to external specialist laboratories. Provision of molecular testing locally would significantly improve both accuracy and timeliness of diagnosis in these cases.

What are the new tests?

AusDiagnostics HighPlex technology for the "Respiratory Virus Panel" was introduced by Viapath's Virology laboratory in December 2015 for simultaneous detection of nine different respiratory viruses. One year later an additional assay was introduced using the same technology but for the detection of seven non-viral pathogens in the lower respiratory tract of severely ill patients, including *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydophila psittaci* and *Pneumocystis jiroveci*.

December 2016 also saw Viapath's Microbiology laboratory introduce a rapid molecular influenza and respiratory syncytial virus (RSV) test, using Cepheid's GeneXpert sample-to-answer technology. This platform enabled the provision of a 24/7 service producing results within 2 hours of the sample being received in the laboratory.



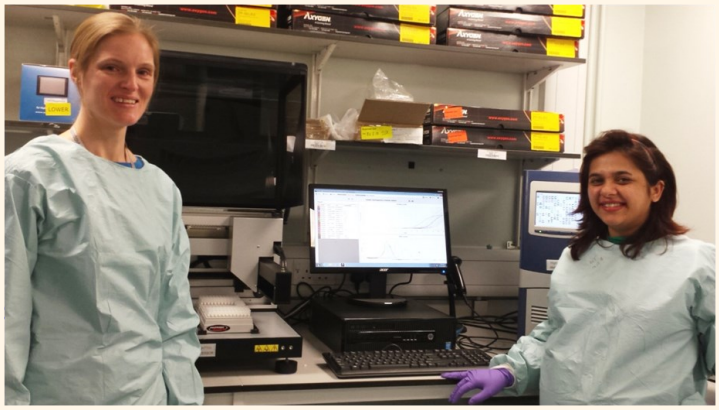
Emma Cunningham (Clinical Scientist) and Liz Nakoneczna (Biomedical Scientist) with the GeneXpert platform

"The provision of a same day result has been positively received by clinics with vulnerable patients as contact tracing and prophylaxis can be sorted out more efficiently. It has also allowed a more intelligent use of side rooms, enabling the "unblocking" of bays during high pressure winter periods." Dr Sam Douthwaite, Consultant Virologist.

There will be a further update in respiratory virus testing in April 2017, with the introduction of a new panel which includes *M. pneumoniae* and *Bordetella pertussis* (the causative agent of whooping cough) plus eight respiratory viruses on the AusDiagnostics platform. Samples from both the upper and lower respiratory tract can be tested using this assay, making it a particular improvement for the timely diagnosis of *B. pertussis* and *M. pneumoniae* infections.

How have these assays improved diagnosis? What is the clinical impact?

Introduction of these three new assays has increased the number of important respiratory pathogens that can now be rapidly detected from routine clinical specimens in our own laboratory. Local provision of molecular assays for detection of pathogens causing non-viral respiratory infections and the introduction of the GeneXpert platform for urgent influenza testing has significantly improved turnaround times for these assays. The clinical benefit of these new assays includes the ability to stop unnecessary use of antibiotics earlier, better targeting of therapy for unusual infections, better use of limited isolation facilities and potentially better outcomes for patients. It is also helpful to provide a definitive diagnosis early so that clinicians don't spend time and resources performing additional diagnostic tests. This is particularly beneficial for patients in the Severe Respiratory Failure Centre in the Intensive Care Unit, as ICU Consultant Nick Barrett explains: "Within the severe respiratory failure population in intensive care, especially those requiring Extra-Corporeal Membrane Oxygenation (ECMO), history and examination are virtually useless in providing clear direction for diagnosis. Hence we rely on a broad brush of rapid, accurate diagnostics in order to provide the optimal therapy to the patient as rapidly as possible. For this population, access to rapid PCR tests for viruses, typical bacterial and atypical pathogens is essential to give the right therapy at the right time. The right diagnosis allows early escalation/de-escalation of antimicrobials and the appropriate use of personal protective equipment and isolation facilities, the latter being a relatively scarce resource. Rapid diagnostics being in routine use improves the care for our patients and better application of resources."



Penny Cliff (Clinical Scientist) and Sweety Tulcidas (Biomedical Scientist) with the AusDiagnostics platform.

Test	Technology	Sample types accepted	When test is offered	Turnaround time
Xpert Flu	Cepheid GeneXpert	Swabs (nose, throat, nose & throat), bronchoalveolar lavage, nasopharyngeal aspirate	Winter "flu season"	4 hours
Respiratory Panel: Standard	AusDiagnostics HighPlex	Swabs (nose, throat, nose & throat, post-mortem), bronchoalveolar lavage, nasopharyngeal aspirate, endotracheal secretions	All year	Next day
Respiratory Panel: Lower	AusDiagnostics HighPlex	Bronchoalveolar lavage, non-directed bronchoalveolar lavage, induced sputum	All year	Next day

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Email: **Emma.Cunningham@viapath.co.uk**

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1. Public Health England (2016). Surveillance of influenza and other respiratory viruses in the United Kingdom: Winter 2015 to 2016.

2. Cunningham, E., Cliff, P., O'Shea, S., MacMahon, E. (2016). Utility of the AusDiagnostics HighPlex Platform for Diagnosis of Respiratory Viruses and Atypical Pneumonia Pathogens. ECCMID ePoster EV0111.

Viapath Celebrates Rare Disease Day

A summary of the day's events from Rosalind Bray, Senior Clinical Scientist .

Rare Disease Day is a worldwide event, the main objective of which is to raise awareness of rare diseases to the general public and members of the healthcare profession, and to demonstrate the impact that these diseases have on patients' lives. Viapath's Inherited Metabolic Disease (IMD) laboratory, at St Thomas' Hospital, deals with rare diseases on a regular basis. It was therefore a great opportunity to get involved with this day and highlight the importance of what Viapath does in the context of patients' lives.

Phenylketonuria

Phenylketonuria (PKU) is one of the rare diseases that the IMD Laboratory is routinely involved in diagnosing and monitoring. Around 1 in 10,000 babies a year are born with PKU which is now predominantly diagnosed through the newborn screening programme. Patients with PKU are not able to break down the amino acid phenylalanine, which then accumulates in the blood and becomes toxic to the brain. Without treatment, patients with PKU can suffer from behavioural difficulties, eczema, recurrent vomiting, tremors and epilepsy. However, since the introduction of the newborn screening programme in the 1960s, patients who are diagnosed with PKU are put onto a strict phenylalanine-free diet which allows the majority of children to lead healthy lives.

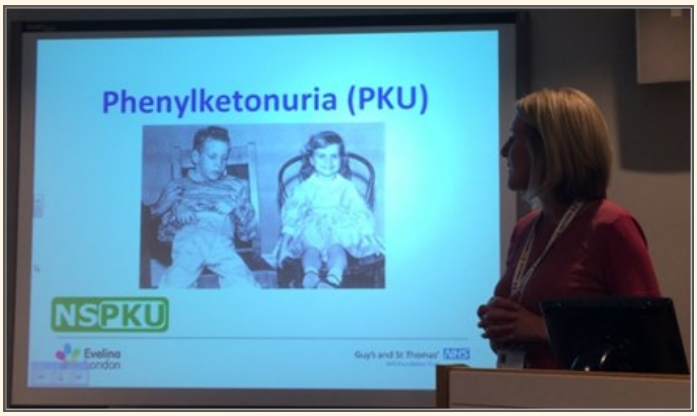

Figure 1 - Arlene Slabbert starts her talk with an introduction

PKU Monitoring

An important aspect of the life of a patient with PKU is monitoring the levels of phenylalanine in their blood, to ensure that they are within the recommended limits. During childhood, patients will send a sample on a weekly basis to check their phenylalanine levels and, if necessary, make changes to their diet. The IMD laboratory carries out the PKU monitoring assay on a daily basis. The assay uses blood from a bloodspot sample which patients can take themselves at home and then send to the lab in the post. The bloodspot is analysed on a mass spectrometer and the patient results are then emailed to the metabolic dieticians. We aim for all cards booked in by 10am to be reported by 4pm on the same day. This allows the dieticians receiving the results to act on them within 24 hours and ensure any necessary dietary changes can be made with immediate effect.

Rare Disease Day Events

1 in 10,000 babies are born with PKU every year

To mark Rare Disease Day at Viapath an event was organised which focussed on PKU. Arlene Slabbert, a metabolic dietician from the Evelina Children's Hospital, spoke first about what happens to PKU results once they leave the laboratory. She explained the importance of receiving the results in a timely fashion so that they can be acted on as soon as possible, with the aim to contact all patients within 24 hours of the result being available. There can be up to 50 patient results on a single day; therefore Arlene described how the youngest patients and those with results outside the recommended limits are prioritised. Depending on the patient's trend of results, different actions will be taken. For example, if the phenylalanine has been outside of recommended limits for two or more consecutive weeks, a dietary change may be required. Since the early days of PKU screening, the dietary options have increased dramatically with many low protein products now on the market.

Arlene described an example food diary for a PKU patient, including carefully measured portions of natural protein containing foods which can then be enhanced with phenylalanine-free products. The patient must also take amino acid supplements to ensure they do not become deficient in other amino acids. Attendees were able to try these supplements, and had to imagine what their breakfast or lunch might look like if they had to adhere to the PKU diet.

Figure 2 - Some information booklets and food supplements available for patients

Following on from Arlene's talk, Caroline Bridges spoke about her personal experience of PKU, as her daughter Hannah was diagnosed 17 years ago following routine newborn bloodspot screening. Caroline described her experience of discovering that her daughter had PKU and the impact that it had in the early days after her birth. It was devastating for Caroline to hear that her child had this rare disease and there was a great deal of uncertainty around what it meant for her and her family. However, she emphasised how important it was that Hannah was diagnosed and treated so early. Caroline described the family's personal experience of coping with the diet and some of the products that work well for Hannah. The good news is that Hannah has been able to achieve fantastic GCSE results and is currently a County and Regional swimmer. It was eye-opening to see the personal impact of a rare disease like this, and with laboratory work being non-patient facing, it was great to be able to put the results that the laboratory generate into perspective.

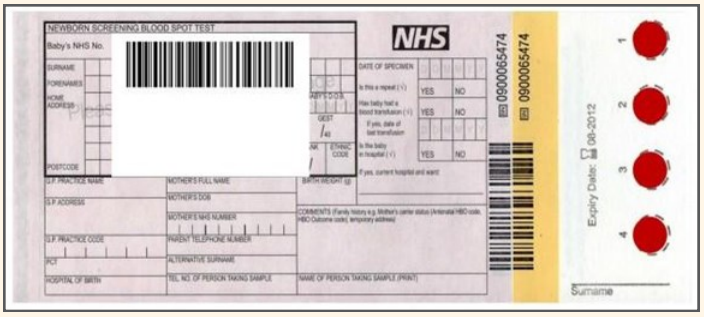

Figure 4 - Bloodspots are collected at home by the patient or their family and sent to the laboratory in the post

Following the talks, tours of the Newborn Screening and IMD laboratories were given to Arlene, Caroline and Viapath colleagues to show them the 'behind the scenes' process. Viapath hopes to hold similar events in the future to reinforce the context of what is done from a patient perspective and to ensure that there is always a reminder of why we do the job we do.

Figure 3 - Arlene gives her talk on how the dietician's are involved in treating and managing patients with PKU

Figure 5 - Ali Lubulwa tries some phenylalanine -free protein supplement

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References

1. Rare Disease Day: http://www.rarediseaseday.org/

2. National Society for Phenylketonuria: http://www.nspku.org/

The Importance Of Using An Acceptable Linear Calibration Curve For Coagulation Factor Assays

Haemostasis:

Haemostasis is the balance of maintaining blood in a fluid state within blood vessels, limiting blood loss after trauma and the removal of blood clots in the process of wound healing. In an healthy status, haemostasis tends towards an anticoagulant state to maintain blood flow and prevent inappropriate thrombus formation. However after tissue injury, an intricate series of events are activated, involving platelets and specific blood proteins (known as coagulation factors) to promote clot formation and prevent excessive blood loss. Knowledge of haemostasis is important in understanding major disease states associated with thrombosis, such as venous thromboembolism (VTE or blood clots), atherothrombosis (thrombosis triggered by plaque rupture) or cardioembolic stroke. Haemostatic disorders can develop for many different reasons and can be congenital or acquired in nature.

Clinical details:

The complex interplay of cellular and molecular components of haemostasis achieves a crucial yet fine balance of procoagulant and anticoagulant mechanisms. Deficiency or defect of any of the coagulation proteins disrupts clot formation and can give rise to a bleeding or thrombotic disorder, depending on where the defect occurs.

Coagulation factor assays are performed to determine the level of a particular factor. This will indicate if a patient has sufficient coagulation activity to maintain haemostasis.

Coagulation factor assays are performed to determine the level of a particular factor which will indicate if a patient has sufficient coagulation activity to maintain haemostasis. A deficiency in any of the coagulation factors is associated with reduced clot formation and excessive bleeding, leading to the dysregulation of haemostasis. One or more coagulation factor activity tests may be ordered to evaluate the function of specific factors.

Accurate determination of clotting factor activity is essential in understanding why a patient may present with a prolonged activated partial thromboplastin time (APTT) or prothrombin time (PT). The screening tests employed to determine the haemostatic potential of each patient will include PT, APTT and Fibrinogen in the first instance, followed by mixing studies as a guide to whether the cause of prolongation is likely to be a factor deficiency, clotting factor inhibitor or lupus anticoagulant. Second line testing will then include intrinsic or extrinsic one stage clotting factor assay or inhibitor analysis, as appropriate.

Description:

One-stage clotting assays for determining coagulation factor function are based on the ability of dilutions of patient's plasma to correct the clotting time of a plasma that has normal levels of all clotting factors except the one being measured, in which it is totally deficient. Clotting times are derived via prothrombin time reagents for factors II, V, VII & X, and APTT-derived reagents for factors VIII, IX, XI, XII, prekallikrein and HMWK. Results are assessed from a parallel line bioassay graphical plot against that of standard plasma.

Figure 1: Sysmex CA-1500

Results:

It is crucial to scrutinise dose response curves in order to establish a central linear component with acceptable differences between plasma dilutions. Also at least three dilutions of test plasma are needed to determine linearity and parallelism. The coagulation data must be checked to confirm the slope ratio or R squared on the print out but also by looking at the shape of the curve. By using the parallel line method to determine relative factor potency this may show evidence of inhibition (most commonly because of the presence of antibodies to coagulation factors or lupus anticoagulants). A point to point curve or a single point result can give a misleading result and inhibitors can be missed.

Figure 2: Clare Dunsmore, Senior Biomedical Scientist

Conclusion:

The importance of using a linear calibration curve was demonstrated in a recent factor IX NEQAS survey (sample 15.24). When using the current linear parallel line curve the results achieved were 117.3, 122.2 and 125.3 u/d L for the 1/1, 1/2, and 1/4 MDA dilutions respectively (mean 121.6 u/d L). Investigation revealed loss of linearity below approximately 15% factor IX activity, leading to subsequent protocol amendments which included altering the initial sample dilution from 1/5 to 1/20 and additional dilutions added to the calibration curve. Re-analysis of NEQAS sample 15.24 gave a mean of 104.8u/dL which would have been a Grade A EQA result.

For further assay results and information on the importance of using an acceptable linear calibration curve for factor assays, ***click here***

One-Stage Factor Assay Service at Viapath

The one-stage factor assay is offered at Viapath's Diagnostic Haemostasis & Thrombosis laboratory, based at St Thomas' Hospital (http://www.viapath.co.uk/our-tests/factor-assays-one-stage-clotting-fii-fv-fvii-fviii-fix-fx-fxi-fxii-pk-hmwk). A Sodium citrate (light blue lid) sample is required and approximately four factor assays can be carried out from each 3.5mL sample. The turnaround time is 7 - 10 days.

One or more coagulation factor activity tests may be ordered to evaluate the function of specific factors

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References/Further Reading

Haematology (Fundamentals of Biomedical Science) 1st Edition by Dr Gary Moore, Gavin Night & Andrew Blann (2010).

Under The Microscope: Dermatopathology

The Dermatopathology Laboratory at Viapath is known as a centre of excellence for dermatological specimens. The laboratory is integral to The St John's Institute of Dermatology, which is one of the world's leading centres for patients with skin diseases and provides support to Guy's and St Thomas' Hospital's plastic surgery department and to GPs with skin-related investigations.

As a specialist laboratory, it processes over 14,000 routine cases a year; including approximately 3,000 complex referral cases from across the UK and internationally. The laboratory's fully automated immunocytochemistry service performs over 24,000 tests annually and employs a panel of over 120 antibody tests as well as supporting over 1,000 provisions of Mohs micrographic surgery.

The laboratory has expertise in Mohs and slow Mohs micrographic surgery techniques, which is an extremely effective procedure that involves investigating the removal of skin cancers by performing extensive section cutting of fresh frozen tissue in order to demonstrate more precise clearance of tumour margins. These skin cancers are most commonly basal cell carcinomas, squamous cell carcinomas and lentigo maligna melanomas and are found mainly, although not exclusively, on facial sites. The Mohs micrographic surgery has a cure rate of 95% to 98%, depending on anatomical site.

Mohs micrographic surgery has a cure rate of 95% to 98%, depending on anatomical site

Although the laboratory deals with skin cancers predominantly, it also performs extensive histological investigations into alopecia and trichogram assessments for patients with hair loss disorders. Alopecia is a common problem, but the root causes are not always understood. This growing specialism is one that generates interest nationally.

The department has a keen interest in both lymphoma and melanoma diagnostic and research work. It is a major referral centre for cutaneous T cell lymphoma, receiving referrals from all over the country.

The department also supports an active research interest in melanoma, assessing prognostic indicators and supporting assessments of treatment strategies, such as BRAF investigations supporting molecular assessments of paraffin wax tissue curls of patient blocks.

The laboratory has a reputation for innovation and scientific endeavour. For example, the laboratory patented a new histological product to improve the process of dealing with hard tissue samples and manufactured this through an external company for use in histopathology laboratories both nationally and internationally.

No laboratory would function well without a well-trained and dedicated workforce. The department has 15 scientific staff with a full spectrum of grades, from band 2 MLA to band 8 BMS. The laboratory is led by Dr Guy Orchard, chief examiner for Cellular Pathology for the Institute of Biomedical Science as well as being an assessor for Immunocytochemistry UKNEQAS and UKNEQAS Cellular Pathology Technique (CPT) and Lead for Mohs. Guy is also a lecturer and short course coordinator.

Figure 1: Members of the Dermatology Laboratory Team

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