Issue 7: August 2017

pathology@viapath

Inside this issue

Message from the Editor

This edition of *pathology@viapath* reminds me of the motto of the Scout Association: "Be prepared". Much of what is done in pathology laboratories requires preparation, whether that be when assisting laboratories in Myanmar to set up testing for childhood cancer or when developing any new tests and test procedures at home. In this edition you will also read that, at present, many scientists are preparing and planning presentations and posters for the IBMS Congress. Viapath has also just introduced an apprenticeship scheme to improve employee development. Above all, pathology may have to be prepared for the unexpected! Pathology departments need to be able to react to events and situations, and a significant amount of work goes into planning laboratory procedures which can be put into action when a major incident happens. Recently Viapath was involved in a simulation exercise to hone such procedures, which proved to be invaluable as recent tragic events unfolded in London. I hope you find our report gives insight into the key role pathology played and reassures you of the preparedness of the emergency services.

Clinical utility of PIVKA-II in the diagnosis of hepatocellular carcinoma

Hepatocellular carcinoma

Primary liver cancer is the third most common cause of death from cancer and the majority of cases are associated with hepatocellular carcinoma (HCC). When diagnosed with HCC, two thirds of the patients have already progressed to the advanced stage of the disease, resulting in a low survival rate. This means that the need for cost-effective and reliable early diagnostic tools is of great interest particularly as an early diagnosis followed by preventative treatment, such as liver re-section or transplantation, can markedly improve prognosis for these patients.

The use of ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI) scans are essential in both diagnosis and assessment of the disease. However, the application of HCC-specific tumour markers, such as alpha-fetoprotein (AFP) and protein induced by vitamin K absence/antagonist-II (PIVKA-II), can be used as supplementary analysis to aid the diagnosis and monitoring of confirmed HCC cases. AFP is currently the most common marker used; however, it has been shown to lack both sensitivity and specificity. Hence, the novel tumour marker PIVKA-II is becoming a more attractive alternative with its proven high sensitivity and greater specificity for HCC.

The nature of PIVKA-II

PIVKA-II is an abnormal form of prothrombin, a precursor protein made in the liver in the presence of vitamin K, that plays a central role in the blood-clotting process. Under normal conditions, prothrombin undergoes post-translational modification, a process where particular enzymes add functional groups to a protein to determine its final structure and, hence, its function. Prothrombin is subjected to a post-translational γ-carboxylation reaction by the γ-glutamyl carboxylase enzyme. However, in the absence of vitamin K, the enzyme is unable to function. Thus, PIVKA-II is formed which ultimately leads to the loss of the biological activity of prothrombin. Interestingly, in some cases carboxylation may not occur at all, resulting in the formation of PIVKA-II variants that each display different degrees of biological activity.

Figure 1. Graphic illustration of PIVKA-II. The red dots represent GLU residues in the GLA domain of the prothrombin (circled area). (©Abbott Laboratories, reproduced with permission)

The role of PIVKA-II in the pathology of HCC

Although the relationship between PIVKA-II and the occurrence of HCC is still not well defined, it has been shown that PIVKA-II can promote an increased rate of cell proliferation as it has the ability to mimic the structure of hepatocyte growth factor (HGF). HGF has the primary function of regulating the growth of hepatocytes (liver cells); however, in the presence of a mimicking protein like PIVKA-II, this can result in uncontrolled hepatocyte proliferation, a signifying characteristic of HCC. Furthermore, PIVKA-II promotes angiogenesis (the formation of new blood vessels) in HCC, resulting in tumour invasion of liver tissue as well as metastases (secondary malignant tumour growths). HCC metastasis occurs via the stimulation of vascular endothelial growth factor (VEGF) which allows the formation of vasculature to supply the growing tumour with oxygenated blood, and epidermal growth factor (EGF) which enables tumour cells to grow, proliferate and differentiate.

Use of the PIVKA-II test to determine presence of HCC

Method

In this study, 87 samples were analysed for PIVKA-II from three groups of patients:

- Group A – randomly-selected patients with non-HCC pathology of the liver such as viral cirrhosis and hepatitis
- Group B – patients with changes to the liver suggestive of possible HCC discovered in the course of US/MRI/CT investigations
- Group C – patients with diagnosed HCC at different stages, where diagnosis was established in the course of histological examination of liver biopsy samples

The analysis was performed by chemiluminescent microparticle immunoassay (CMIA) using a two-step sandwich reaction where a binding reaction occurs between antibodies against PIVKA-II and specific binding sites (epitopes) on PIVKA-II in the patient sample, followed by a washing stage, with the subsequent addition of chemiluminescent labels, resulting in the emission of light. This enables the quantification of PIVKA-II concentration in the tested sample.

Results

In the positive group, PIVKA-II was elevated in 85.3% of the patients; normal PIVKA-II levels detected in this group can be explained by the normalisation of PIVKA-II concentration after treatment (Table 1). In contrast, an elevated PIVKA-II concentration was found in just one patient from the negative control group; this result may be interpreted as either false positive (elevation of PIVKA-II due to non-HCC pathology) or true positive (the patient would need to undergo more comprehensive screening to confirm the presence of HCC).

Control group	Percentage of elevated PIVKA-II results (%)	Percentage of elevated AFP results (%)
Negative control group (high-risk patients with non-HCC pathology)	3.4	16.7
Positive group (patients diagnosed with HCC at different stages)	85.3	70.6

Table 1. Positive PIVKA-II and AFP results in negative and positive control groups

Marker	True positive	True negative	False positive	False negative	Truly reliable
PIVKA-II (*n*=63)	27/63, 42.9%	28/63, 44.4%	1/63, 1.6%	7/63, 11.1%	**>83%**
AFP (*n*=51)	24/51, 47.1%	14/51, 27.5%	3/51, 5.9%	10/51, 19.5%	**75%**

Table 2. True/false positive/negative result analysis

In this study, the analysis revealed that more than 83% of PIVKA-II results were reliable, in comparison with 74.6% of AFP results showing true diagnostic value (Table 2). Taking into account considerable difference between sensitivity and specificity rates for PIVKA-II and AFP (79.4 vs 96.6% and 70.6 vs 82.4% respectively), allows the conclusion that PIVKA-II displays slightly better clinical utility in HCC diagnosis.

Conclusion

PIVKA-II has several advantages over AFP in terms of clinical utility for HCC diagnosis and prognosis: it is more sensitive to small HCC tumours, correlates with HCC progression significantly better and has a shorter half-life than AFP, making it more suitable for monitoring the cancer. However, there are some limitations; one of these being its vulnerability to the action of potentially interfering pharmacological agents such as warfarin and certain antibiotics. Therefore, the combination of PIVKA-II and AFP is suggested to be the best option for highly accurate laboratory diagnosis of HCC, supplementary to imaging techniques.

For further information, please contact:

Human Nutristasis Laboratory: 0207188 6815 / 89543

Acknowledgement:

This article has been adapted, by Parmilla Dhamrait, from a paper written by Volha Klimovich, Kieran Voong, Roy Sherwood and Dominic Harrington that appeared in Clinical Laboratory, June 2017

References

McMasters K, Vauthey J. Hepatocellular carcinoma: targeted therapy and multidisciplinary care. Springer 2011; Chapters 1–5, 8.

Ji J, et al. Diagnostic evaluation of des-gammacarboxy prothrombin versus α-fetoprotein for hepatitis B virus-related hepatocellular carcinoma in China: a large-scale, multicentre study. PLoS One 2016; 11: e0153227.

Lim TS, et al. Combined use of AFP, PIVKA-II, and AFP-L3 as tumor markers enhances diagnostic accuracy for hepatocellular carcinoma in cirrhotic patients. Scand J Gastroenterol 2015; 51: 344–353.

De J, et al. A systematic review of des-γ-carboxy prothrombin for the diagnosis of primary hepatocellular carcinoma. Medicine 2016; 95: e3448.

Kokudo M, et al. Evidence-based clinical practice guidelines for hepatocellular carcinoma: The Japan Society of Hepatology 2013 update (3rd JSH-HCC Guidelines). Hepatol Res 2015; 45: 123–127.

Childhood cancer in Myanmar (Burma)

Childhood cancer in Myanmar: the story so far

Nearly 3,000 children in Myanmar are expected to develop cancer each year. There are two centres that can provide treatment; Yangon Children's Hospital and Mandalay Children's Hospital. However, due to a lack of awareness about childhood cancer among the public and healthcare professionals, an inability to accurately diagnose cases and the absence of funding for treatment-related costs, the number of newly diagnosed cases is far lower than expected. In addition to this, many families take their children home early before finishing treatment because of the financial burden related to long stays in hospital.

The main problems Myanmar faces with childhood cancer:

- Little or no specialist training for healthcare professionals
- Poor diagnostic facilities
- Late diagnosis of children presenting with an advanced stage of disease
- Limited psycho-social care for patients' families or staff

Improvements that can be made include:

- Development of a reliable electronic childhood cancer database
- Development of a training programme for healthcare professionals
- Subsiding treatment costs
- Reduce abandonment of treatments through psycho-social support and education for families, as well as the provision of accommodation, food and transport

Immunohistochemistry (IHC) training November 2016

Immunohistochemistry training

One of the key activities proposed by World Child Cancer (WCC), an organisation committed to improving the rate of diagnosis, accessibility of treatment and quality of support for children with cancer in the developing world, was to improve diagnostic techniques and training. In response to this, in October/November 2016, a training course on Immunohistochemistry (IHC) was organised in collaboration with the Yangon Children's Hospital and Viapath's Cellular Pathology Department at St Thomas' Hospital. Participants benefited from discussions on case studies, access to guidelines, journals and web resources throughout the training course. They were also taught how to implement good practices using the existing laboratory materials and equipment.

The participants agreed that the course was very relevant for guiding day-to-day practice and that the documentation would be a useful resource for reinforcement and further enhancement of the training of health care providers.

Dr Robert Carr and Ruth Sardinha (far right) with training attendees April 2017

Training results

The results obtained from the workshop were beyond initial expectations. The trainees showed excellent technical and scientific competency and, one week after the training, the Histopathology laboratory at Yangon Children's Hospital were able to perform IHC independently and support the Histopathology diagnosis.

The participants realised that the role of partnership is essential in supporting education and training in order to promote improvement in diagnosis and patient treatment management. It could be made through:

- Implementation of best practice guidelines and education
- Identification of training needs
- Continuous professional development

However, an effort should be made to guarantee:

- Training and support for medical and biomedical staff on essential histology techniques, necessary for cancer diagnosis and treatment management
- Establishment and support for education and training in specialised techniques, such as ancillary tools of histopathology diagnosis
- Support for inter-institutional collaboration between the different diagnostic laboratories/ hospital in Yangon
- Support for the implementation of Health & Safety measures in diagnostic laboratories
- Collaboration with the other healthcare institutions (national and international) for e-learning and educational support

Future plans

New efforts have been made by World Child Cancer to continue the project. A second phase will include training and support to Mandalay healthcare institutions with the aim of further improving access to quality diagnosis and care for more children.

In April 2017 another visit to Myanmar was made. The principal aim was to further build a partnership between visiting Viapath staff and the Yangon Children's Hospital team and to apply the learning from this partnership to assess the state of Paediatric Oncology in Mandalay. WCC's intention is to extend the support effort over the coming 3 years on training and improving infrastructure for the Paediatric Oncology service in Mandalay and middle Myanmar.

For further information, please contact:

Ruth Sardinha Cellular Pathology 02071882911

Ruth.Sardinha@viapath.co.uk

To read more about World Child Cancer go to:

https://www.worldchildcancer.org/

World Child Cancer
No child should suffer

The multi-disciplinary approach for the diagnosis of multiple myeloma

Multiple myeloma is the malignant growth of plasma cells that accumulate in the bone marrow, leading to bone destruction and marrow failure.

Plasma cells play a central role in the immune system. They develop from B-cells (a type of white blood cell) and produce large amounts of immunoglobulins (antibodies). Multiple myeloma occurs when abnormal plasma cells escape control and expand rapidly beyond the constraints of normal cell death. The most common symptoms include bone pain, recurring infection, kidney damage and fatigue. The acronym CRAB, shown in Figure 1, is used to identify the main presenting features.

Calcium elevation

Renal complications

Anaemia

Bone disease

Figure 1: CRAB - the main presenting features of multiple myeloma

Survival rates in myeloma are increasing at the fastest rate among all cancer types in the UK

What causes myeloma?

The causes of myeloma are not fully understood but it is thought to develop due to an interaction between both genetic and environmental factors.

Myeloma usually evolves from an asymptomatic pre-malignant stage of clonal plasma cell proliferation termed monoclonal gammopathy of undetermined significance (MGUS). MGUS is present in more than 3% of the population above the age of 50 and progresses to myeloma or related malignancy at a rate of 1% per year.

Investigation	Result
Serum calcium	Hypercalcaemia (elevated calcium) occurs in 30% of patients with myeloma
Full blood count (determines the type and number of cells in the blood)	Normocytic and normochromic anaemia is present in 80% of patients (meaning patients have red blood cells that are of normal size and contain a normal haemoglobin concentration, respectively, but are insufficient in their number)
Creatinine and urea (indicators of renal function)	Renal impairment is present in 50% of patients and is associated with a worse prognosis
Albumin (the main protein found within serum)	Low albumin is used as a prognostic marker
Beta-2 microglobulin (a membrane protein which is elevated in multiple myeloma)	High beta-2 microglobulin is used as a prognostic marker
C-reactive protein (a marker for inflammation caused by infection or disease)	Higher levels indicate more extensive disease and is associated with a worse prognosis
Lactate dehydrogenase (an enzyme that can be used to monitor disease progression)	Higher levels indicate more extensive disease
Bone marrow aspirate and biopsy	Helps differentiate multiple myeloma from MGUS and solitary plasmacytoma (a build up of abnormal plasma cells in the bone)
Serum free-light chain assay (measuring the immunoglobulin light chains that circulate freely in serum)	Diagnostic and monitoring test, and used for follow-up examination of non-secretory multiple myeloma
Imaging: Skeletal survey, MRI and CT scans	Detects osteopenia (decreased bone density), osteolytic lesions (severe bone loss which appear as "holes" on an X-ray and pathological fractures)
Serum/urine electrophoresis (analysis of the amount of different proteins within serum/urine)	Diagnostic test for multiple myeloma
Cytogenetics and Fluorescent in situ hybridisation (FISH)	Specialist genetic testing for chromosomal abnormalities associated with prognosis

Laboratory tests

A multidisciplinary approach is important for the diagnosis of multiple myeloma, with laboratory tests from Biochemistry, Haematology, Immunology and Genetics all providing key results.

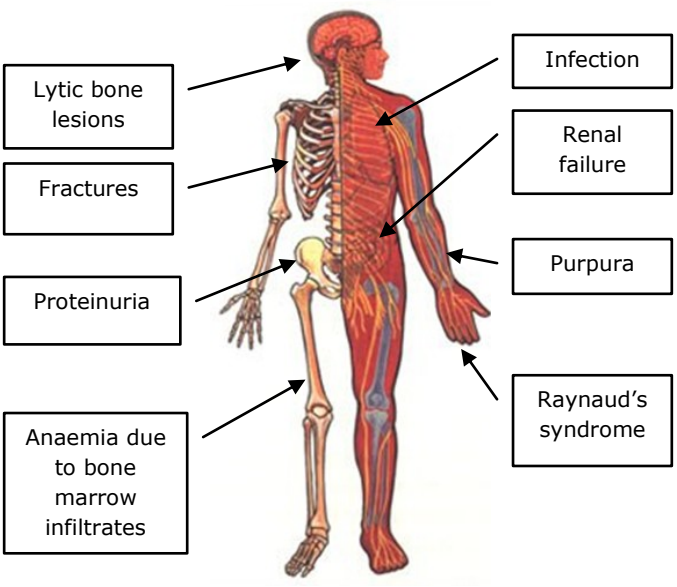

Figure 2: Multiple myeloma is characterised by specific symptoms related to the clonal process

Diagnostic tests for multiple myeloma

Electrophoresis:

Protein electrophoresis separates the proteins in a blood sample into several groups based on their electrical charge and size. Albumin is the major protein component of serum and appears as the biggest peak that lies closest to the positive electrode. Globulins comprise of a much smaller fraction of the total serum protein but are the primary focus of interpretation. The gamma fraction lies closest to the negative electrode.

Figure 3 shows the protein categories in a normal trace compared to the trace of a myeloma patient. In the latter, a dense, narrow band composed of a single type of immunoglobulin can be seen in the gamma region. This is secreted by an abnormally expanded clone of plasma cells and is known as a monoclonal protein (M-protein, paraprotein). Highlighting the area of abnormality, and looking at the percentage of total protein this area represents, allows quantification of the protein to be given in g/L.

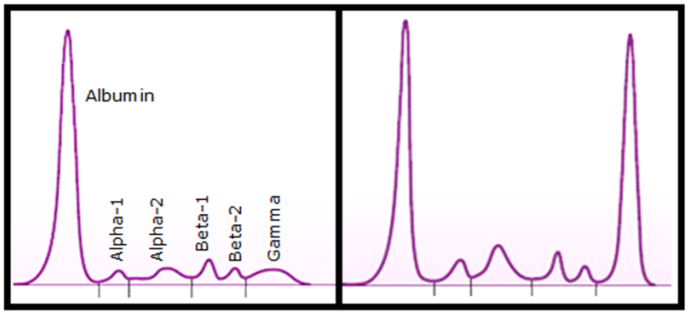

Figure 3: Protein categories in a normal serum electrophoresis trace (left) and abnormal monoclonal protein (right) seen in a Myeloma patient

Upon detecting an abnormal monoclonal protein, immunofixation can be used to identify the heavy and light chain of the clonal immunoglobulin (Ig). In this method, serum samples are electrophoresed in agarose gel and then specific antibodies are added to the gel to bind to corresponding proteins before staining the gel. Visual identification of the heavy chain (IgG, IgA, IgM, IgD, and IgE) and the corresponding light chain (kappa, lambda) is then possible. Figure 4 shows an example of an immunofixation gel, with the applied antisera, containing the specific antibodies, labelled above each.

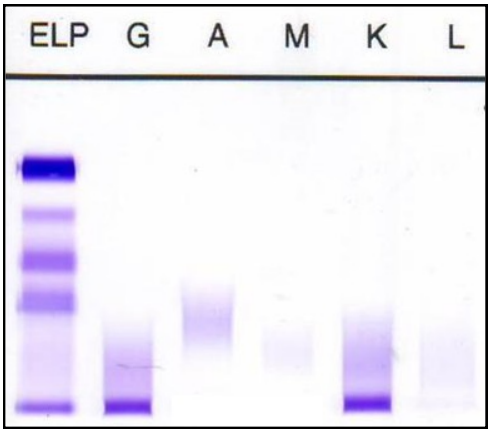

Figure 4: IgG kappa monoclonal protein identified by immunofixation in a patient with myeloma. ELP= Total protein stain, showing albumin at the top through to the gamma region at the bottom of the gel

Fluorescent in situ hybridisation (FISH):

FISH uses fluorescent probes that bind to specific regions of the cell's DNA to identify gains, losses and rearrangements of the genetic material.

The plasma cells in bone marrow samples are positively selected for the CD138+ markers on the outside of the cells. These plasma cells are collected and processed to allow hybridisation of the FISH probes, as shown in Figure 5.

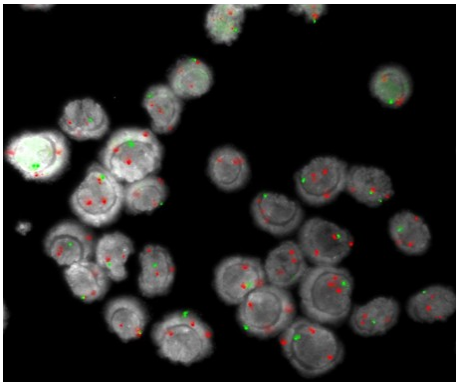

Figure 5: Fluorescent microscope image showing plasma cells with gain of 1q (red) and loss of 1p (green)

The results provide an indication of the prognosis. Clinicians use this information to help with the diagnosis and also aid in treatment regimens for the patient.

Abnormalities include:

Hyperdiploidy (the occurrence of additional chromosomes to the normal diploid set)	• Occurs in ~50% of MM • 48-74 chromosomes • Usually gains in odd numbered chromosomes (3, 5, 7, 9, 11, 15, 19 and 21)
Monosomy 1 (only one chromosome is present from a homologous pair) or 13q deletions	• Found in 30-50% of cases
Chromosome 1p (short arm) and 1q (long arm) abnormalities	• Found in 30-40% of cases • Including: -1q whole arm or partial duplication -1p deletion
TP53 (17p13) Deletions	• Found in ~10% of cases • Associated with complications including hypercalcaemia, renal failure and CNS
IGH@ 14q32 rearrangements	• Translocation of Ig enhancers to oncogenes that are located on different partner chromosomes, leading to their upregulation • Translocation partners: - Cyclin D1 (11q13) - C-MAF (16q23) - FGFR3/MMSET (4p16.3) - Cyclin D3 (6p21) - MAFB (20q11)

Treatments available

Improvements made to treatment over the last decade have meant that survival rates in myeloma are increasing at the fastest rate among all cancer types in the UK.

In the past decade, increased use of proteasome inhibitors (drugs that prevent the activity of proteasome complexes that break down proteins) and immunomodulatory drugs (which are able to suppress the immune system) have improved the outlook for myeloma patients; however the disease still remains incurable. Triple drug combinations are routinely used in induction regimes, followed by autologous stem cells transplantation in appropriate patients as shown in Figure 6.

Other treatments will also be prescribed to help prevent or manage potential side-effects and treat the symptoms and complications of myeloma. The final goal should be to find a balance among efficacy, toxicity and cost as well as the dream of achieving the cure for this disease.

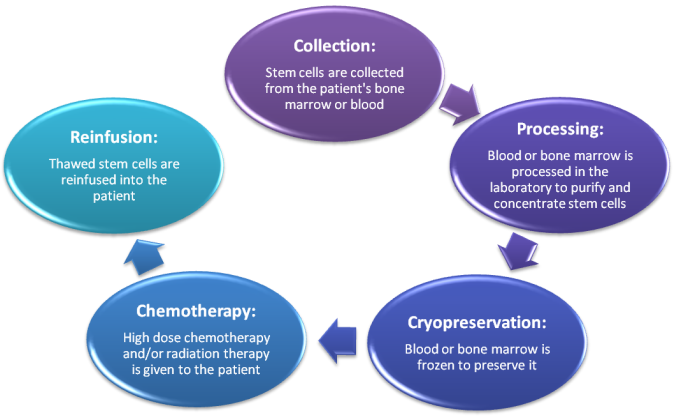

Figure 6: The process of autologous stem cells transplant

For further information regarding the genetic tests, please contact:

www.viapath.co.uk/departments-and-laboratories/genetics
Genetics Department
5th Floor Tower Wing
Guy's Hospital
Great Maze Pond
London
SE1 9RT

Sample requirements for genetic tests:
Cytogenetic analysis in plasma cell myeloma is carried out by interphase fluorescence *in situ* hybridisation (FISH) analysis of CD138 positive cells enriched from bone marrow aspirate samples.
Sample type: bone marrow aspirate in bone marrow transport medium. Do not spin down or freeze samples before sending. Samples must arrive within 24 hours.
Current service turnaround times: 5-10 calendar days (National targets: Non-urgent - 95% reported within 21 days)

For more information about the immunology tests, please contact:

kch-tr.immunology@nhs.net
Diagnostic Immunology and Allergy Department
King's College Hospital
Bessemer Wing - 1st Floor
Denmark Hill
London
SE5 9RS

Sample requirements for immunology tests:
Serum/urine protein electrophoresis, Immunofixation/ Immunotyping serum free kappa and lambda light chains.
Sample type: clotted (gold top vacutainer)
Volume: 4mL
Turn around time: 3 days (Immunofixation 1 week)

Apprenticeships within pathology

Viapath has responded to the Government's new Apprenticeship Levy scheme, by working with "3aaa Apprenticeships" to set up the VIAcademy. This initiative not only creates new apprenticeship positions but also provides existing employees with exciting training and development opportunities that are relevant to their job roles.

Mirali Patel was recently hired as an Apprentice Facilities and Logistics Administrator and has embarked on her apprenticeship journey through 3aaa Apprenticeships' King's Cross training.

She commented on her first week in her role;

"I have been welcomed into Viapath and am enjoying the challenge. I'm looking forward to continuing to meet more experienced and knowledgeable people so that I can grow and develop professionally and become a skilled member of the Viapath team."

Already Mirali is being joined by more apprentices all of whom will be developed through the Apprenticeship Levy in Service Support, Customer Service and Learning & Development roles.

In addition, over 60 Viapath employees from across the organisation – including laboratory and corporate employees – will be coached through the Apprenticeship Levy 3aaa Apprenticeships' Ofsted Outstanding accredited training courses. This means that all employees, whether new to Viapath or an existing member, have the opportunity to further develop themselves and learn from our and others' expertise.

Mary Fitzgerald, Viapath's HR Director, commented on the work that has taken place:

"Through the VIAcademy, we're in a great place to utilise the funds that the Government requires us to pay into the Apprenticeship Levy and I have been delighted by the response both internally from our own workforce and externally from the potential apprentices that we are interviewing. A number of people in our HR, Finance and Communications teams have worked very hard alongside our Apprenticeship Levy Partners, 3aaa Apprenticeships to get the VIAcademy up and running. Collectively we see our work with 3aaa Apprenticeships as the start of a lasting relationship to develop both our existing workforce, as well as to help new talent onto the first rung of their career ladder."

Figure: Left to right; Michael Holder (Viapath), Mirali Patel (Viapath), Rakhee Patel (Viapath), Jeff Bowcok (3aaa Apprenticeships), Michelle Plange (Viapath)

Major incident simulation & planning

Planning for major incidents from a pathology perspective.

All laboratories providing acute services to the NHS have major incident plans which are carefully worked through strategies detailing the laboratory, team and individual responsibilities. They involve an element of second-guessing what, who and when resources will be needed and then attempt to build these into practice, ready for any eventuality. The overall objective is to provide reassurance that, in the event of a major, mass casualty event, every cog in the wheel will work as expected, to deliver the right standard of care, at the right time, independent of demand and time of day.

David Wells, Viapath's Operations Director for Reference Services, explains: "Having sat through many desk-top exercises, it was a rare opportunity to be able to run through a full major incident, not only with more than one agency, but also 'live' with a fully operational laboratory and hospital. When the Viapath Laboratories at St Thomas' hospital were approached to play their part in this exercise, Viapath's Blood Transfusion Team jumped at the chance."

Jess Child, Resilience Manager at Guy's and St Thomas' NHS Foundation Trust, picks up the story. "Exercise Unified Response" was a Europe-wide exercise that was run by the London Fire Brigade in March 2016. It provided the opportunity to test the Trust's Major Incident/Mass Casualty Plan with a live simulation."

The scenario

The exercise was based on a mass casualty incident, caused by a building collapse at London Waterloo train station, resulting in buried tube trains and requiring casualty extraction by urban search and rescue teams.

This high-profile exercise was attended by all of London's emergency services. The exercise was set in the disused Littlebrook Power Station in Dartford, which was remodelled as London Waterloo station with 2,000 volunteers participating. Over four days of live play, 1,100 casualties were extracted from the scene and flowed into a notional hospital and disaster victim mortuary. As part of the planning, the Resilience Management Team worked with key players, including Viapath's pathology laboratories, to ensure all aspects of the trauma pathway management were involved and their corresponding action cards tested and validated.

Realism was key

On March 2nd, a group of casualties, with agreed injury loading, played by paramedic students from the Institute of Pre-Hospital Care at London's Air Ambulance/Queen Mary's University London, flowed into the Emergency Department (ED). Six core patients were picked for evaluation and other patients were also added to the scenario. These included patients requiring code red blood transfusion, abdominal trauma, pregnancy and attending uninjured "relatives" who required support. Realism was key, so the casualties had simulated trauma injuries. The air of realism was further achieved by the Trust's Simulation and Interactive Learning Centre whose role was to facilitate the use of patient monitoring to simulate clinical observations, which required the clinicians to make real-time decisions to manage clinical presentation.

Jess' Resilience Management Team played a key role in maintaining continuity of ED service during the exercise, with the lead facilitator based at the ambulance doors to maintain exercise timings and pause live play when a real emergency patient required access.

Figure 1: The emergency services coordinating their response

It was very important not to disrupt the normal activity of ED and robust communications were key in this process. All ED patients were informed that a live exercise was taking place and walk-in patients were given leaflets. The Resilience Management Team also worked closely with the trust communications team to ensure information about the exercise was disseminated appropriately.

The blood bank

As part of the planning team, Tim Maggs, Viapath's Blood Transfusion Laboratory Manager at Guy's and St Thomas', explains the laboratory's role. "We were approached about taking part and were really keen to fully test the laboratory's ability to manage the diagnostic and transfusion demands during a major incident. There was no attempt to alter the 'business as usual' level of processing and samples from real

patients and these continued appropriately. Continuous review of the department's ability to cope was undertaken by observers to ensure patient care wasn't compromised".

"In essence, the blood bank's initial response to the incident involved the deployment of a 'mobile blood bank' containing boxes of universal donor blood to the ED department, which was managed by the nominated Blood Transfusion Coordinator. This allowed instant dispensing of universal donor red cells for immediate red cell replacement and, importantly, a mechanism through which all subsequent orders for blood components were placed in the ED. The Transfusion Coordinator liaised with the central blood bank for supply of plasma and platelets and also for re-supply of universal donor blood. In the meantime, the blood bank was redeploying teams, reviewing and replenishing stock and clearing any blood requests to prepare for patients as they flowed through to theatres and intensive care units."

"In order to make the exercise as real as possible, a ghost blood stock was created on the non-live environment of the laboratory informatics system. Units of red cells, plasma, platelets and cryoprecipitate were photocopied and the 'paper' units deployed. As requests came in, the paper units were issued, boxed and crashed to the requesting locations. During a busy night in the blood bank, you could get three code red patients simultaneously over a 12-hour shift. During the exercise, we were exposed to six simultaneous code red patients – all before lunch time!"

Figure 2: Rescuers at the scene

The debrief

Tim explains the debrief process. "Although the laboratory responded extremely well, there were useful learning opportunities for the team involved on the day and relating to wider process, equipment and environment evaluation.

"Once the exercise had been stood down, we gathered our team for a debriefing session. From a blood bank point of view, the most challenging aspect of a major incident is clear and concise communication between the clinical teams and the laboratory, and establishing a single point of contact. While within the laboratory, keeping track of urgent requests for multiple patients is extremely challenging."

The Resilience Management Team also captured patient experience feedback during the exercise. Every "patient" in the exercise was followed through their journey by a clinical facilitator who audited the patient's care and met with them to discuss their experience at the end of the exercise. Feedback was very positive with patient dignity, reassurance and clear explanations of what was happening described as being well managed. It was noted that the ability of the Trust to run the exercise live, through the working ED with realistic casualty simulations and with control rooms fully operational, added significantly to the understanding of major incident response, command and control, and trauma management.

David Wells says: "From the laboratory's point of view, this exercise was a visual interpretation of our major incident plan, bringing it to life, showing who we would be liaising with, how often, and when. It also demonstrated how our processes would work, what the impact would be upon the routine service, and most importantly, how effective we would be in the event of a mass casualty event. Working with our partners, we have found it incredibly reassuring to be able to put our plan into action and thoroughly test it, it's the plan that we all hope we will never need to use, but if we do, we know we are ready." He concludes: "As one of the observers during the event, it was humbling to see the professionalism, dedication and aptitude of the individuals who might one day save your life."

Jess Child – Resilience Manager, Guy's and St Thomas' NHS Foundation Trust

Tim Maggs – Blood Transfusion Laboratory Manager, Viapath, Guy's and St Thomas' Hospital

David Wells – Director of Operations, Reference Service, Viapath, Guy's and St Thomas' and King's College Hospitals

Footnote from David Wells:

On March 22nd and June 3rd 2017, the events on Westminster Bridge and London Bridge brought home the need to practice our major incident plan. Viapath teams put into place their training calmly and professionally, teams remained at their posts (even those who had personally witnessed the events unfold) or headed from other sites to support colleagues where needed. Everyone, without fail, contributed to ensure an effective and high quality service to those in need.

Faster approach to detecting common bacterial gastrointestinal pathogens by multiplex PCR in faecal samples

Common gastrointestinal pathogens that cause "food poisoning" are very prevalent in the UK. Food poisoning can cause symptoms such as vomiting, diarrhoea, stomach cramps and fever depending the bacteria or virus causing the infections. Generally, food poisoning may occur after eating undercooked food e.g. chicken, incorrectly stored and prepared food, eating food washed in contaminated water i.e. salad leaves and from eating food prepared by someone who already has the bacteria. Actually the term "food poisoning" is a bit of misnomer as many cases of bacterial gastrointestinal infections are spread by other routes of transmission that are not necessarily food related. These include: person-to-person spread, transmission through contact with animals e.g. children contracting E.coli O157 when visiting petting farms, water contaminated with human or animal faeces and insect vectors e.g. flies.

For the UK, all food poisoning bacteria isolated in microbiology laboratories are reportable to the government public health organisations. The most frequently isolated bacterial food pathogens are Campylobacter spp, Salmonella spp, Shigella spp and Escherichia coli O157. Figure 1 below show the number of cases of common bacterial gastro-intestinal infections in the UK in 2015 and 2016 for England, Wales, Scotland and Northern Ireland.

Public Health Region	**Campylobacter**		**Salmonella**		**Shigella sonnei**		**E.coli O157 (verotoxin producing)**	
Year	2015	2016	2015	2016	2015	2016	2015	2016
England and Wales	56277	52129	7924	7563	925	703	700	814
Scotland	6264	5296	803	836	70	*No data	183	*No data
Northern Ireland	1320	*No data	125	*No data	16	*No data	33	*No data

*No data available at the time of writing this article

Figure 1: Incidence of Campylobacter, Salmonella , Shigella and E.coli O157 in the UK in 2015 and 2016.

Figure 2: Image of Salmonella ssp (a common cause of the food poisoning in the UK) growing on Xylose Lysine Deoxycholate agar

Faster detection by Multiplex Polymerase chain reaction (PCR)

From August 2015 to August 2016, before PCR was introduced, the Microbiology Laboratory at St Thomas's Hospital London, received 8000 specimens for faecal culture from both GP and hospital patients. Culture methods for detecting these gastro-intestinal pathogens, involved the culture of the faeces onto culture plates and into broth i.e. Xylose Lysine deoxycholate (XLD) and selenite broth for Salmonella and Shigella, Sorbitol MacConkey for *E.coli* O157 and blood free campylobacter agar for Campylobacter. After an incubation of up to 48 hours, any "suspicious" bacteria that might be causing the infection are selected from the other non-pathogenic bacteria found in faeces. These then undergo further identification, using the MALDI-TOF (matrix assisted laser desorption and ionisation – time of flight) spectrometry, biochemical tests and serology before the microbiology clinician is informed of a gastrointestinal infection.

In the summer of 2016, Viapath Infection Sciences - Microbiology Laboratory, in collaboration with the Centre for Clinical Infection and Diagnostics Research (CIDR), introduced multiplex PCR technology. This is used to detect four common causes of gastrointestinal infection in faecal samples from hospital patients (patients in hospital <3 days) and GP patients. Other gastro-intestinal infections caused by agents such as Vibrio spp, Yersinia spp and parasites are not currently detected by PCR in this laboratory, so other laboratory techniques are still used.

CIDR with Viapath validated the multiplex PCR using the Becton Dickinson Max (BDMAX) analyser (see figure 3 below). Small amounts of faecal samples are placed into the PCR cartridges with the reagent strips and placed onto the BDMAX. Time from processing the sample to the final PCR result takes just under 3 hours. All PCR results are reported immediately through our LIMS (Winpath) system and any positive samples for Salmonella, Shigella and E.coli (vero-toxin producing) are subsequently cultured for species specific serology and antibiotic sensitivity testing. PCR negative samples and samples positive for Campylobacter are not cultured (as the illness is usually self-limiting and can be treated with antibiotics for severe cases without significant concern of antibiotic resistance).

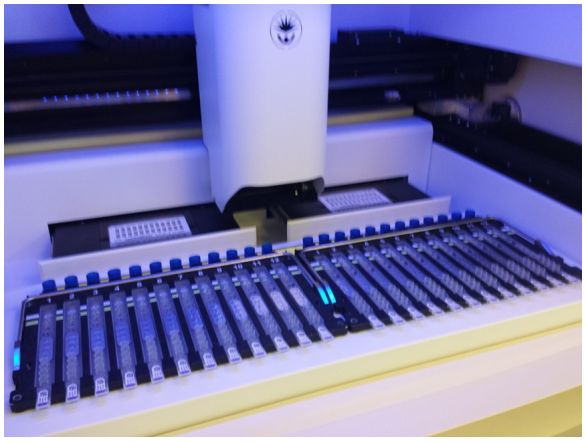

Figure 3: BDMAX and the PCR cartridges – 24 tests per run

Figure 4: BDMAX in action

The laboratory has found this new PCR platform fits in well to the laboratory workflow, has dramatically reduced the turnaround times for the results and significantly reduced the numbers of faecal cultures performed.

Impact on patient care and Infection Control

Prior to the introduction of this molecular panel, the laboratory turnaround time for detection of these pathogens was 66.5 hours which meant that often the patient had been discharged from hospital by the time the result was known. After introducing this molecular panel, the laboratory turnaround time was reduced to just over 3 hours, meaning that by performing two runs daily a same day result could be provided for all patients. As well as improving sensitivity, the faster turnaround time has allowed much better management of patients, particularly in relation to infection control. As most positives are also cultured and sent on to the reference laboratory, we can maintain the useful information gained from culture for public health and epidemiology purposes.

For more information about this test please contact

Lisa Bryan, Microbiology Operations Manager 02071883114

Lisa.bryan@viapath.co.uk

Lyndsay Rowley, Senior Biomedical Scientist 02071883111

Lyndsay.rowley@viapath.co.uk

Dr Simon Goldenberg FRCPath, HipHIC, MD Consultant Microbiologist and Infection Control Doctor, Guy's and St Thomas' NHS Foundation Trust, London

In the spotlight: Viapath Customer Services

Viapath recognises the importance of delivering exceptional customer service to its customers. With this in mind, Viapath recently decided to expand its Customer Service department to develop the service offered.

Over the coming months, the team will be introducing new processes and implement improvements. There are now two dedicated teams that users may contact, one is situated at St Thomas' hospital and the other at King's College hospital. They will be able to answer queries about results, deal with requests to perform additional tests on an existing sample, respond to questions related to courier collections and deal with any ad hoc issues that may arise. Other enquiries related to pathology may also be sent to the team; if they cannot answer the request they will pass it onto the relevant department.

The team will acknowledge all requests within 24 hours of being received (Mon-Fri, excluding Bank holidays).

The teams may be contacted at:

St Thomas' Hospital

Tel: 020 7188 8008

Email: customersupport@viapath.co.uk

King's College Hospital

Tel: 020 3299 3576

Email: Viapath.customersupport@nhs.net

Head of Customer Services Rupinder Gill said "this is an exciting time and provides a great opportunity to grow and develop the current service we offer to our customers."

Customer Service Team left to right Khalifa Salim, Alfeeza Ladak, Caitlin Formon, Suphia Khatun, Oana Poraicu

Viapath presentations at the Institute of Biomedical Science's Congress

The Institute of Biomedical Science IBMS CONGRESS 2017 24-27 SEPT - BIRMINGHAM, UK

The Institute of Biomedical Sciences is holding its biennial congress on September 24th to 27th at the International Conference Centre in Birmingham. Viapath is proud to have several speakers and poster presentations. Here is a selection:

LECTURES

Monday, September 25th: Opening Plenary Session

Transformation and Consolidation: a partnership model

Professor Jonathan Edgeworth, Medical Director

Providing a pathology service presents many challenges as well as key opportunities; these will be discussed within the context of Viapath's changing environment.

Monday, September 25th: Clinical Chemistry

Screening and Diagnosis of Inherited Metabolic Disorders

Erin Mozley, Biochemical Sciences

Inherited Metabolic Disorders (IMDs) are an expansive heterogeneous collection of diseases caused by genetic mutations affecting an enzyme, transporter or other protein involved in a metabolic process. Three of the core biochemical tests for screening of IMDs in patients are urine organic acids, plasma amino acids and bloodspot or plasma acylcarnitines. The analytical, interpretative and clinical aspects of these assays will be discussed, as well as an insight into the vast number of more specialised tests that are available to help diagnose and monitor IMDs. In newborns, a national screening programme uses bloodspots to enable early diagnosis, and therefore earlier treatment, of six inherited metabolic disorders. These disorders will be discussed in more detail, as well as tandem mass spectrometry, the powerful technique used across the world to screen for IMDs in newborns.

Tuesday, September 26th: Clinical Chemistry

How to Validate Real-Time PCRs

Dr Melvyn Smith, Virology Department

The real-time polymerase chain reaction is one of the core technologies in the diagnosis of infectious diseases. The early stages in the development of the technique were followed by a dramatic increase in the number of diagnostic assays being published, followed by the introduction of commercially produced tests. In this presentation some of recent work covering verification and validation methodology will be presented, together with the impact of ISO15189 and UKAS inspections on the process, to provide a practical and standardised experimental approach.

Tuesday, September 26th: Cellular Pathology

A comparison of BRAF V600E immunohistochemistry and molecular screening in 71 cases of malignant melanoma

Karolina Wojcik, Dermatopathology Laboratory

The BRAF mutation activates the protein and the downstream Map Kinase (MAPK) signalling pathway, this promotes proliferation of tumour cells and subsequent spread. Here we report the use of a monoclonal antibody BRAF V600E (Roche Diagnostics) which detects the main BRAF mutation in metastatic malignant melanoma (MM) in 71 patients in comparison to the existing molecular assay.

New embedding and staining systems PrestoCHILL and Presto stainer for evaluation of cryostat tissue in Mohs micrographic surgery: A step forward for automation in frozen section analysis.

Cristina D'amico, Dermatopathology Laboratory

Mohs micrographic surgery (MMS) conventionally involves the evaluation of frozen histological tissue sections to determine complete circumferential and deep tissue margin clearance of cutaneous skin tumours. In the large majority of cases these tumours are basal cell carcinomas (BCC's), the most common form of skin cancer. PrestoCHILL and Presto stainer devices are two new innovative tools which bring benefits of automation, speed and efficiency for the preparation of histological frozen section analysis in Mohs procedures. The devices were assessed at Viapath's Tissue Science Mohs laboratory, St. John's Institute of Dermatology at Guy's Cancer Centre.

Haematoxylin - the story of the blues!

Dr Guy Orchard, Dermatopathology Laboratory

Despite the advent of synthetic dyes, the use of haematoxylin for staining tissue has endured to the present. This presentation explores the history and uses of this versatile organically derived stain.

Mohs UK NEQAS CPT pilot EQA scheme: Exciting developments on how to land the plane successfully!

Dr Guy Orchard, Dermatopathology Laboratory

The introduction of a new pilot scheme for Mohs was created as a result of a defined need across the UK for an EQA scheme that previously hadn't existed and in addition the need for evidence based participation in an established EQA scheme required by UKAS and ISO 15189 standards for laboratory practice. The new scheme encompassed several training sessions for all the assessors in order to establish the correct level of expectation of performance of all the participants.

Wednesday, 27th September: Haematology

Beyond Textbook Diagnosis

Dr Gary Moore, Diagnostic Haemostasis & Thrombosis Laboratory

Many disorders of haemostasis and thrombosis can be expected to manifest in predictable patterns with panels of laboratory assays and diagnosis being relatively straightforward. Rare sub-types of some disorders may not conform to standard presentations and can go undiagnosed without further tests and informed interpretation.

Wednesday, September 27th: Immunology

Autoimmune bullous dermatoses – case studies

Dr John Mee, Immunodermatology Laboratory

Case presentations which highlight how our tests can help in diagnosis of these patients.

Wednesday, September 27th: Cellular Pathology

Skin Antibodies

Dr John Mee, Immunodermatology Laboratory

An overview of the diagnostic techniques offered and how the results can be used in diagnosis and monitoring of patients.

POSTER PRESENTATIONS

Monday, September 25th

Age and sex-specific ferritin reference intervals for iron status assessment

Nadia Munim, Diagnostic Haemostasis & Thrombosis Laboratory

A modified Hoffmann's approach was used to establish age and sex-specific ferritin ranges. Both lower and upper limits for this marker are clinically important, since low values suggest deficiency leading to anaemia, and high values may reflect iron overloading/acute phase. Therefore, accurate and subgroup-specific reference intervals should be applied. Application of these reference intervals will aid iron status assessment and support patient care.

Tuesday, September 26th

TruSlice and TruSlice Digital histological dissection devices, introducing an exciting development in providing improved accuracy and precision at the cut- up bench

Mohammad Shams, Dermatopathology Laboratory

TruSlice and TruSlice Digital are two new innovative tools which enable all the dissection factors to be controlled. Both devices are based on a guillotine configuration, one with plastic inserts (TruSlice) and the other with an electronic micrometre attached (TruSlice Digital). The devices were assessed in 5 hospitals and the precision and reproducibility was evaluated.

Wednesday, September 27th

Zinc transporter 8 antibodies: To test or not to test

A Rhodes & M Peakman: Clinical Immunology Laboratory

Zinc transporter 8 (ZnT8) is a pancreatic β-cell secretory granule membrane protein that has been newly identified as a target of humoral immunity in type 1 diabetes. The measurement of autoantibodies to insulin, glutamic acid decarboxylase 65 and protein tyrosine phosphatase IA-2 by radioimmunoassay encompass our current testing strategy for type 1 diabetes mellitus (T1DM). However, ZnT8 has been shown to be more specifically expressed in insulin-containing secretory granules than that of GAD and IA -2. This study analysed the prevalence of ZnT8 antibodies in routine T1DM testing and considered the benefit of including ZnT8 in our testing panel to improve diagnostic accuracy.

Incident, response and outcome: A study involving Bronze Commander responses to multiple incidents affecting pathology services at Viapath Analytics outside of core hours

Dervilla Gorman, Haemostasis & Thrombosis Laboratory

A Gold-Silver-Bronze command structure is used by Viapath Analytics at Guys and St Thomas' NHS Hospital Trust to establish a hierarchical framework for the command and control of major incidents and disasters that could affect the pathology service, health and patient care. This poster highlights the varied types of incidents that have taken place, how they were resolved and the final outcome. It also highlights the importance of having a Bronze commander on-site to deal with these incidents and their role as a single point of contact, to facilitate the provision of all pathology.

Second tier screening test for raised C5 newborn screening samples

D Burden, K John & R S Carling: Biochemical Sciences

Following a pilot study, the national newborn screening programme was expanded to include screening for Isovaleric Acidaemia (IVA). This is performed by flow injection analysis mass spectrometry; however in addition to detecting the compound of interest, isovalerylcarnitine, it also detects isobaric compounds including pivaloylcarnitine. Data from the pilot study demonstrated a high number of false positive results and it is well documented that exogenous sources of pivaloylcarnitine can be responsible for this. A method was set up to chromatographically separate the isobaric compounds in order to identify the predominant compound present in true and false positive newborn screening samples.