Issue 2

pathology@viapath

Message from the Editor

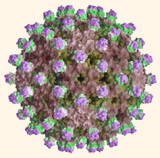
Welcome to the second edition of pathology@viapath, updating you with information from Viapath's scientists, the tests that are being run and the research that is being undertaken.

If you missed the first edition of this newsletter, it is still available to view on Viapath's website and includes items on Zika virus, genetic testing, porphyria and testing for drugs of abuse.

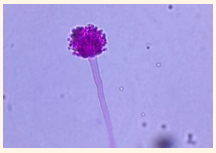
I hope you find these newsletters interesting. However, if there are any topics that you would like to be covered in future editions of pathology@viapath, please get in touch.

Inside this Issue:

Case Study : Vitamin B_{12} Deficiency.



Hepatitis E Virus: An Emerging Zoonosis.



Improving the Diagnosis of Invasive Fungal Infections in Critically Ill Patients.

Why And How Should Vitamin D_2 and D_3 Be Measured?

WORLD THROMBOSIS DAY
13 OCTOBER

Why And How Should Vitamin B_{12} Be Measured?

Case Study: A Baby with Severe Vitamin B_{12} Deficiency

A nine month old girl was admitted to the Metabolic Unit at Evelina Children's Hospital for the assessment of developmental delay, abnormal movements (head drops forward and arms move up) and macrocytic anaemia with massive excretion of urinary methylmalonic acid. Further tests revealed severe vitamin B_{12} deficiency. The mother was also diagnosed with vitamin B_{12} as well as iron deficiency. The girl had been exclusively breast fed from birth. The introduction of solids at 5 months was unsuccessful because of feeding difficulty.

Maternal B_{12} deficiency, most likely masked by iron deficiency and a normal haemoglobin count on ante natal screening, led to severe B_{12} deficiency in the baby. Exclusive breast feeding and subsequent failure to wean exacerbated the B_{12} deficiency leading to profound functional deficiency of B_{12}. Appropriate clinical treatment was given and remarkable improvements in the baby's health were observed.

To read the full case study, click on this link or for further details on B_{12} testing visit our website
For further information contact Dr Agata Sobczyńska-Malefora on 020 7188 9543 or at agata.malefora@viapath.co.uk

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Hepatitis E Virus: An Emerging Zoonosis

Hepatitis E: The Disease and the Virus

The hepatitis E virus (HEV) is a small virus, with a positive-sense, single-stranded ribonucleic acid (RNA) genome, that infects both humans and animals. Most individuals with HEV are asymptomatic and the infection clears completely. However, in immunocompromised patients the HEV infection may persist, potentially leading to chronic hepatitis and cirrhosis.

'1 in 3,000 blood donors in the South of England had an HEV viraema at time of donation.'

HEV can cause sporadic hepatitis (an inflammation of the liver) as well as large outbreaks of hepatitis and is an emerging zoonosis, for instance, crossing the species barrier from pigs to humans. Hepatitis E is widespread in Southeast Asia, northern and central Africa, India and Central America, with an increasing number of acute HEV infections being seen in Europe.

The virus is mainly spread by the faecal-oral route and the most common route of infection in the UK is from eating raw or undercooked meat, especially pork and shellfish. However, HEV can also be transmitted by blood products and a study carried out recently showed that approximately 1 in 3000 blood donors in the south of England had an HEV viraemia at the time of donation. This is likely to be an underestimate. On this basis, the Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) has recommended that certain groups of immunocompromised patients, such as those undergoing solid organ transplantation and haematopoietic stem cell transplantation, should receive HEV negative blood components as well as monitoring for HEV infection

New Test for the Hepatitis E Virus

Viapath has developed a quantitative HEV RNA real time reverse transcription polymerase chain reaction (RT-PCR) assay specifically to monitor the presence of the virus in susceptible groups of immunocompromised patients and this test will be offered as a new diagnostic service in October 2016.

In addition, Viapath will also provide HEV IgM and IgG antibody detection to help make the diagnosis of either an acute or past HEV infection in patients who may be jaundiced or have deranged liver function tests.

For further information on the scientific or clinical aspects of Hepatitis E, please contact:

Dr Mel Smith: melvyn.smith@nhs.net
Dr Mark Zuckerman: mark.zuckerman@nhs.net

Or for information on the testing procedures for Hepatitis, please contact:

Fearghal Tucker: fearghal.tucker@nhs.net

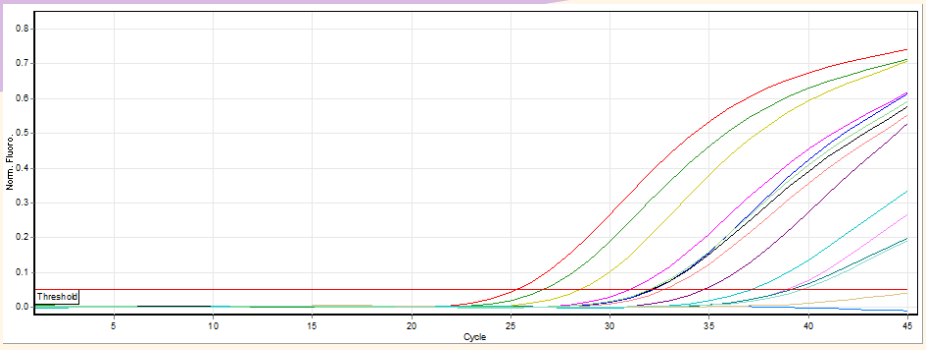

Figure 1: A typical HEV reverse-transcription PCR assay run on the Rotor -Gene showing amplification of the five quantification standards and eight samples. The horizontal red line (Threshold) set at 0.05 represents the point at which exponential amplification begins. This is the cycle at which quantification values for each sample are calculated, based on the cycle threshold values (Cts). The lower the cycle number the more viral RNA was in the extract before the RT-PCR began.

Improving the Diagnosis of Invasive Fungal Infections in Critically Ill Patients.

Invasive fungal infection is a major success-limiting factor in modern healthcare, especially for the immunocompromised and those in the critical care unit setting. Yeasts such as *Candida albicans* and *Candida glabrata* are implicated in deep-seated infections - including bloodstream infections - in Critical Care Unit patients, and the mould *Aspergillus fumigatus* in the immunocompromised such as solid organ and stem cell transplant recipients.

Although yeasts are often recovered from blood cultures, generally fungal infection – and aspergillosis in particular - goes undiagnosed or diagnosis is delayed as cultures are persistently negative.

'Yeasts are implicated in deep-seated infections.'

A delay in diagnostics worsens prognosis. Critically ill patients may receive expensive antifungal drugs empirically to provide cover for these infections, and these drugs are not without side-effects.

To improve the diagnosis of invasive fungal infections in critically ill patients, Viapath offers a test for Beta-D-glucan (BDG). BDG is a cell wall molecule produced by both yeasts and filamentous fungi, such as *Aspergillus* spp. Effectively, it is a 'pan-fungal marker' the presence of which indicates invasive fungal infection, be it yeast or mould. Thus BDG can be used to monitor patients, both those on treatment and those at risk of infection.

BDG is found at very low concentrations in the blood of healthy individuals (10-40 pg/mL) but at concentrations exceeding 80 pg/mL in established fungal infection. The assay has a high negative predictive value: a negative BDG result encourages reconsideration of the need for antifungal therapy in patients receiving such agents.

Viapath's Infection Science Department at King's College Hospital, now uses the CE marked Fungitell assay which is a rapid colorimetric assay for the detection of BDG in the serum of patients with symptoms of, or medical conditions predisposing to, invasive fungal infection.

For patients at prolonged risk of developing invasive fungal infections, samples may be tested twice weekly as a "screening" test. Specimens may also be submitted from patients, in critical care units, with a prolonged pyrexia which is unresponsive to antibacterials and therefore suspected of having a fungal infection. A sample of clotted serum is required for the test and the turnaround time is 36-48 hours.

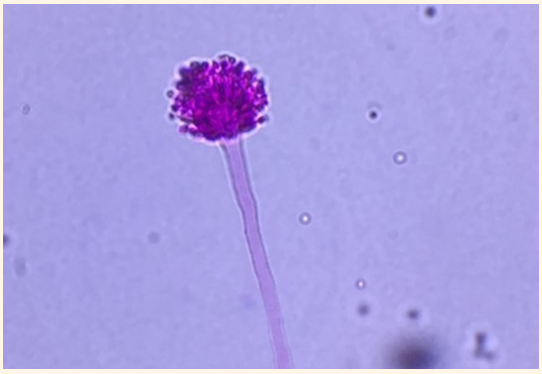

Figure 1: Aspergillus Fumigatus. Courtesy of Dr S.Braham

For further information on Viapath's BDG test, visit Viapath's website here or contact Dr Sharleen Braham
020 3299 2571 or at SBraham@nhs.net

For further information on the clinical aspects of BDG testing, please contact:
Dr Jim Wade: Jimwade@nhs.net
Dr Anita Verma: Anitaverma.nhs.net

Why And How Should Vitamin D_2 and D_3 Be Measured?

What is Vitamin D?

There are several different forms of vitamin D and those that we are specifically interested in are 25-hydroxyvitamin D_2 and D_3 ($25OHD_2$/D_3). Vitamin D_2 is made by some plants, and vitamin D_3 is synthesised in the skin in response to sunlight exposure during the summer months.

The monitoring of vitamin D levels in patients is important for the prevention and control of disease. Its measurement is indicated in cases of suspected vitamin D deficiency e.g. rickets (children), osteomalacia (adults), neonatal hypocalcaemia, nutritional osteodystrophy (especially in the elderly, institutionalised and Asian populations, malabsorption (e.g. in cystic fibrosis, primary biliary cirrhosis) and in patients on long-term anticonvulsant therapy (barbiturate and phenytoin combination therapy in particular).

Vitamin D – Why is it important?

Vitamin D plays a critical role in regulating calcium and phosphorus levels in the body. If these levels are not adequately controlled, bone mineralization conditions, such as rickets in children or osteoporosis in adults may occur. Vitamin D has other roles in the body, including modulation of cell growth, immune function and reduction of inflammation. Recent studies identifying an association for vitamin D in prevention of cancer and cardiovascular disease have generated renewed interest in monitoring vitamin D levels in serum.

Vitamin D can be found in a small number of foods including, oily fish, red meat, liver and egg yolks. The recommended daily intake of vitamin D for adults (>1 Years old) is 10mcg but from late March to September sufficient amount of vitamin D should be synthesised from exposure to sunlight. It has been noted that vitamin D supplements have been linked to halving the risk of an individual suffering from a severe asthma attack.

Requests for vitamin D testing have increased considerably over the last few years leading to a huge demand for robust laboratory assays capable of processing large numbers of samples. The accurate and precise measurement of vitamin D_2/D_3 has been challenging because vitamin D is lipophilic and the presence of the vitamin D binding protein interferes with quantification of $25OHD_2$/D_3.

How does Viapath Test for Vitamin D_2 and D_3?

Over the last 3 years, the Viapath Nutristasis Unit has used an automated MultiPurpose Sampler (MPS)-LC-MS/MS with the ITSP™ (Instrument Top Sample Preparation) as the pre-analytical sample preparation method for the analysis of vitamin D_2 and vitamin D_3 in human serum. The MPS is able to move the ITSP™ device anywhere on the autosampler deck enabling significant flexibility in methodology. Some of the benefits of using ITSP on the MPS include a significant reduction in consumables, solvent volumes, sample volume, time and labour required for sample preparation. The MPS features an automated internal standard addition, protein precipitation stage, automated centrifugation and supernatant handling, as well as miniaturized solid phase extraction (SPE) stage to remove matrix impurities, all coupled directly to LC-MS/MS (Agilent 6460 MS/MS).

This approach enables Viapath's Nutristasis Unit to process a high number of samples since sample preparation now occurs automatically. Moreover, each sample extract is treated in exactly the same way and prepared just before the analysis, improving sample to sample reproducibility when compared to a manual batch process. Finally, sample sequence integration between the sample preparation system and analytical system reduces possible transcription errors.

Vitamin D is converted into 25-OH vitamin D in the liver. This is the major storage form of vitamin D and is the analyte of choice for determination of the vitamin D status.

For testing vitamin D, Viapath requires a Serum Separator Tube (SST) sample and the test has a turnaround time of 1 week. For more details please visit Viapath's website.

For further information, please contact Renata Gorska, Senior Clinical Scientist, Human Nutristasis Unit on 02071886815 or at renata.gorska@viapath.co.uk

Did You Know World Thrombosis Day was on 13th October?

What is World Thrombosis Day?

World Thrombosis Day (WTD), a campaign of the International Society on Thrombosis and Haemostasis, focuses attention on the often overlooked and misunderstood condition of thrombosis.

Professor Beverly Hunt, Clinical lead for Haematological Sciences at Viapath and member of the steering committee for World Thrombosis Day.

'Thrombosis kills one in four people worldwide each year.'

WTD seeks to increase global awareness of thrombosis, including its causes, risk factors, signs/symptoms and evidence-based prevention and treatment. Ultimately, the campaign strives to reduce death and disability caused by the condition and supports the World Health Assembly's global target of reducing premature deaths by non-communicable disease by 25% by 2025, as well as the World Health Organization's global action plan for the prevention and control of non-communicable diseases between 2013 and 2020.

WTD takes place every year on 13th October, the birthday of Rudolf Virchow who was a pioneer in the pathophysiology of thrombosis. Virchow was an inspired German physician, pathologist, biologist and anthropologist, who developed "Virchow's triad - the three risk factors for blood clots - sticky blood, damaged vein walls and reduced blood flow in veins due to immobility. These are the three factors that lead to deep vein thrombosis and pulmonary embolism. The major cause of deep vein thromboses and pulmonary embolism is admission to hospital. We use the term "hospital-acquired thrombosis" for any DVT/PE developing in hospital or for 90 days afterwards. Both King's and Guy's & St Thomas' provide national and international leadership in this area. Indeed NHS England leads the world in its prevention of hospital-acquired blood clots, and the death rate due to pulmonary embolism has dropped by 8% since all English hospitals adopted mandatory prevention.

Why is Thrombosis Awareness Important?

Thrombosis kills one in four people worldwide each year and is the leading cause of global death and disability. It is also the underlying origin of the top three major cardiovascular killers: heart attack, stroke and venous thromboembolism (VTE). A condition that must not be ignored, thrombosis awareness around the world is limited, with most adults not realizing that blood clots can be prevented. Further, VTE awareness is virtually non-existent, providing the opportunity to elevate understanding and focus on prevention and empowerment to reduce unnecessary deaths.

WORLD THROMBOSIS DAY
13 OCTOBER

For more information or to register as a campaign partner visit:
www.worldthrombosisday.org
Or contact Dr. Beverley Hunt at
Beverley.Hunt@gstt.nhs.uk

Thrombosis diagnostics in Viapath

There numerous reasons why someone can have sticky blood, damaged blood vessels or reduced blood flow and patients with more than one of the risk factor categories are at an increased risk of thrombosis. One of the roles of diagnostic laboratories is to assist in diagnosis of the presence of a thrombosis when patients show symptoms of deep vein thrombosis or pulmonary embolism. This is done by measuring a substance that is produced when the body is trying to destroy the clot, called D-dimer. If the result is normal, the patient is experiencing their symptoms for a different reason, but if the level is high, doctors know they need to get medical imaging tests done, such as ultrasound, to see the clot itself so that they can make informed decisions about treatment.

Amongst the reasons for having sticky blood is a group of disorders called thrombophilias where patients have a genetic abnormality in one of the proteins that regulate blood clotting. Viapath's Haemostasis & Thrombosis Laboratories at Guy's & St. Thomas' Hospitals are the largest of their kind in the UK and perform thousands of specialised tests a year that check which of the proteins are present at lower levels than normal or are not functioning properly. If an abnormality is found, the laboratories can also look for the exact change in the DNA that caused the abnormality. Antiphospholipid syndrome is an acquired thrombophilia because the cause of sticky blood is not genetic but because patients make abnormal antibodies that cause thrombosis. There is a different set of specialist tests that detect these antibodies.

Viapath's Diagnostic and Molecular Haemostasis and Thrombosis Laboratories at Guys & St. Thomas' Hospitals are recognised nationally and internationally as expert centres for these specialist tests, and Viapath's Blood Sciences Laboratory at King's College Hospital also performs some of these tests.
The main treatment for thrombosis is anticoagulant therapy to thin blood and another set of tests are used in the laboratories to check that levels of the drugs are not too high or low. Viapath's laboratories at King's are an expert centre for measuring levels of the newer anticoagulant drugs.

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Dr Paradzai Chitongo, Operations Manager & Lead Scientist, Blood Sciences/Haemostasis, KCH; paradzai.chitongo@viapath.co.uk

Some of Viapath's Relevant Publications on Thrombosis

1. Brown SA, Mitchell M, Cutler JA, Moore G, Smith MP, Savidge GF. Rapid genetic diagnosis in neonatal pulmonary artery thrombosis caused by homozygous antithrombin Budapest 3. *Clin Appl Thromb Hemost* 2000;6:181-183
2. Cutler JA, Mitchell MJ, Greenslade K, Smith MP, Savidge GF. A rapid and cost-effective method for analysis of three common genetic risk factors for thrombosis. *Blood Coagul Fibrinolysis* 2001;12:33-36
3. Keeling D, Mackie I, Moore GW, Greer I A, Greaves M. and British Committee for Standards in Haematology. Guidelines on the investigation and management of antiphospholipid syndrome. *British Journal of Haematology* 2012;157:47-58
4. Bramham K, Retter A, Robinson SE, Mitchell M, Moore GW, Hunt BJ. How I treat heterozygous hereditary antithrombin deficiency in pregnancy. *Thrombosis & Haemostasis* 2013:110:550-559
5. Ledford-Kraemer MR, Moore GW, Bottenus R, Daniele C, de Groot PG, Exner T, Favaloro EJ, Moffat KA, Nichols WL. Laboratory testing for the lupus anticoagulant. Approved guideline – first edition. CLSI document H60-A. Clinical and Laboratory Standards Institute Wayne, PA, USA April 2014
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7. Moore GW, de Jager N, Cutler JA. Development of a novel, rapid assay for detection of heparin-binding defect antithrombin deficiencies: the heparin-antithrombin binding (HAB) ratio. *Thrombosis Research* 2015;135:161-166
8. Patel JP, Chitongo PB, Czuprynska J, Roberts LN, Patel RK, Arya R. Normal prothrombin times in the presence of therapeutic levels of apixaban--in-vivo experience from King's College Hospital. *Br J Haematol* 2015;169:152-153
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Why And How Should Vitamin B_{12} Be Measured?

Vitamin B_{12} - What is it?

Vitamin B_{12} (B_{12}), which is more correctly known as cobalamin, plays a vital role in the formation of red blood cells and in the function of the brain and nervous system. It is one of eight B vitamins, the others being B_1, B_2, B_3, B_5, B_6, B_7 & B_9 (the missing numbers are due to the compounds being declassified as vitamins), all of which are involved in cell metabolism. Vitamin B_{12} is involved in the metabolism of every cell of the human body, especially affecting DNA synthesis, fatty acid and amino acid metabolism.

'B_{12} deficiency is common in >10% of people 75 years +'

What are the manifestations of Vitamin B_{12} deficiency?

Risk factors for B_{12} deficiency include restricted dietary intake of animal products, impaired gastric absorption, loss or inactivity of intrinsic factor (*Addisonian* pernicious anaemia), pancreatic insufficiency, impaired intestinal absorption (*e.g.* ileal resection in Crohn disease), multiple congenital factors and acquired drug effects.

B_{12} deficiency is common, with a prevalence of ~5% in people 65–74 years of age and more than 10% in people 75 years of age or older.
The detection and correction of B_{12} deficiency prevents megaloblastic anaemia and potentially irreversible neuropathy and neuropsychiatric changes. Crucially, 20% of B_{12}-deficient patients have no discernable haematological diathesis

Vitamin B_{12} - What are traditional diagnostic tests?

Testing for B_{12} status is problematic because no single laboratory marker is suitable for the assessment of B_{12} status in all patients. Most laboratories estimate B_{12} status by measuring the abundance of B_{12} in serum and comparison against a predefined reference range. However, this approach gives no indication of B_{12} utilisation and it is known that serum B_{12} assays generate a high rate of false-negative results. This means that up to 45% of B_{12}-deficient subjects can be overlooked if only serum vitamin B_{12} assays are used as a screening test. The application of multiple laboratory markers greatly improves the diagnosis of B_{12}-deficiency.

Novel markers of B_{12} status include holotranscobalamin (marketed as 'active B_{12}'). Holotranscobalamin is the form of B_{12} taken up by cells to meet metabolic demand. Laboratory B_{12} status markers that reflect cellular utilisation rather than abundance are also available. In humans, two forms of B_{12} act as coenzymes for two different reactions. Methionine synthase requires methylcobalamin for the remethylation of methionine from homocysteine. A homocysteine concentration >20 µmol/L may suggest B_{12} deficiency in folate-replete patients. In the second B_{12}-dependent reaction, methylmalonyl-CoA mutase uses adenosylcobalamin to convert methylmalonyl-CoA to succinyl-CoA. In B_{12} deficiency excess methylmalonyl-CoA is hydrolysed to methylmalonic acid. A serum concentration >280 nmol/L may suggest suboptimal status in young patients with normal renal function.

Vitamin B_{12} - How is Vitamin B_{12} measured at Viapath?

The Viapath Nutristasis Unit has used holotranscobalamin as the first-line marker for the assessment of B_{12} status since 2012, a two-step immunoassay using chemiluminescent microparticle immunoassay (CMIA) technology. The Unit has found that ~5% of requests from mixed patient populations have a holotranscobalamin <25 pmol/L and are classified as deficient based on this test alone; an indeterminate result of 25–70 pmol/L is measured in ~25% of samples leading to secondary test using methylmalonic acid analysis – of these one in three patients are found to be deficient. All other samples have a holotranscobalamin concentration >70 pmol/L and are classified as B_{12} replete.

If you would like further information then please refer to the recent Best Practice article:
Harrington DJ. J Clin Pathol 2016 http://jcp.bmj.com/content/early/2016/05/11/jclinpath-2015-203502.long

Other Recent Publications Relating to B12 from the Nutristasis Unit

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