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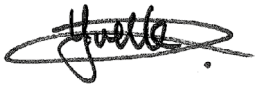
Message from the editor

As we all know, the National Health Service is not just about doctors and nurses but other professions too, who exhibit an equally great diversity of roles and skills. Recently I was privileged to represent Viapath at the Advancing Healthcare Awards 2018 and it was fantastic to see these other professions, including healthcare scientists, being recognised and rewarded for the valuable work that they do. I was impressed to hear words like innovation, leadership, creativity and compassion being used time and again to describe their achievements and to illustrate how these dedicated professionals are taking the Health Service to the next level.

The overall winner's story is truly inspirational. Malcolm Robinson co-founded the charity Harvey's Gang, named after 6 year old in-patient Harvey Buster Baldwin, who was curious about where his blood samples were going. Malcolm gave Harvey an explanatory tour of his laboratory and arranged for him to have a child-size lab coat, cardboard security pass with 'trainee biomedical scientist' written on it and goody bag. When a consultant paediatrician told Malcolm that seven more critically ill children also wanted a tour, he got to work. Since then the charity has organised VIP laboratory tours for more than 200 young patients in 50 locations, including Viapath. You can read about one of these visits in this edition of pathology@viapath. You can also read about the winners of the Viapath award for innovation in healthcare, who developed a virtual reality experience to help prepare paediatric patients for an MRI scan which, for them, can often be a difficult and scary experience.



Improving Diagnostic Services for Unknown Sexually Transmitted Infections

Background

Currently, Viapath's Virology Laboratory at St. Thomas' Hospital provides a service for detection of pathogens causing sexually transmitted infections (STIs) using the Hologic Panther platform. This service, which offers testing for *Neisseria gonorrhoea* (the causative agent of gonorrhoea), *Chlamydia trachomatis* (the causative agent of chlamydia and lymphogranuloma venereum) and *Trichomonas vaginalis* (a causative agent of vaginitis and cervicitis in women, and occasionally urethritis and balanitis in men), will soon be expanded to include testing for *Mycoplasma genitalium*. This is a more recently discovered pathogen, which was first reported in 1981[1] and remains relatively unknown to the general population.

What is *M. genitalium* and why test for it?

M. genitalium is a small, flask-shaped, pathogenic Gram negative bacterium with a cell membrane but no cell wall, and lives on epithelial cells of the urinary and genital tracts of men and women. Although it was first reported in 1981, it was not until 1983 that it was reported as a new *Mycoplasma* species unrelated to any other known *Mycoplasma*[2].

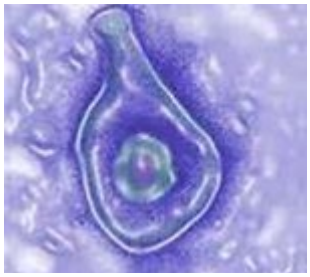


Figure 1: M. genitalium

M. genitalium is a cause of non-gonococcal urethritis in men, and can also cause cervicitis and pelvic inflammatory disease in women. It is the second most common cause of non-gonococcal urethritis in men (behind chlamydia), however prevalence in the general population is currently low (<5%)[3]. Higher prevalence rates are seen in men who have sex with men and those engaging in high risk sexual behaviours. Despite the current low prevalence, this infection has a significant impact as persistence and recurrence are common and approximately 40% of sexual contacts of a case will also test positive for this pathogen. Routine testing has not been widely performed due to the difficulty and time taken for conventional microbiological culture, limited availability of suitable molecular detection assays, and the time and cost associated with reference laboratory testing.

Despite the limitations that have prevented routine laboratory testing of *M. genitalium*, the need for laboratory diagnosis has become more acute, and testing is now recommended in UK National Guidelines[3, 4]. In addition, antimicrobial resistance is becoming increasingly prevalent in *M. genitalium*, with approximately 40% of cases being resistant to macrolides such as azithromycin. A single dose of this antibiotic is the first-line treatment for gonorrhoea[5] (in combination with another antibiotic) and chlamydia[6] as this simple dosing regimen means there is good treatment compliance. This treatment can, however, cause the emergence of macrolide resistance in *M. genitalium*. The most recently published treatment guidelines for non-gonococcal urethritis from the British Association for Sexual Health and HIV (BASHH) now recommend seven days of doxycycline as first line treatment[4], although this regimen has a treatment failure rate for *M. genitalium* of up to 68%[3]. Azithromycin is used as an extended course (lasting 5 days) for the treatment of *M. genitalium* where there is no evidence of macrolide resistance. Where macrolide resistance is present or anticipated, a course of moxifloxacin is advised, but resistance to this is also on the rise. Dual resistance is becoming a problem in Japan and Australia, and could easily become more common in Europe. Alternative antibiotic options are extremely limited if doxycycline, azithromycin and moxifloxacin are ineffective. There is therefore a real possibility that M. genitalium may become the new superbug. In view of this, a test of cure is recommended after 3 weeks to ensure successful clearance of the infection.

What is the new test and how well does the test perform?

The Aptima *M. genitalium* assay uses transcription mediated amplification (a nucleic acid amplification technology or NAAT) to detect the ribosomal RNA in the bacterial cells. As there are multiple copies of ribosomal RNA, this assay is more sensitive than standard NAATs that detect a gene which is present as a single copy in each bacterial cell.

Verification of the assay was carried out in 2017. Specimens from genitourinary medicine (GUM) patients were tested and results compared with those obtained at the reference laboratory. In addition, collaborative work with Brighton and Sussex University Hospitals NHS Trust enabled us to assess performance of the Aptima assay with another commercial assay (FTD Urethritis Plus, Fast Track Diagnostics Ltd., Sliema, Malta). 279 samples were tested. Of these, 160 were from patients attending our local GUM clinic with symptoms of infection, or risk factors for infection. The remaining 119 were samples that had been tested in Brighton using another commercial assay.

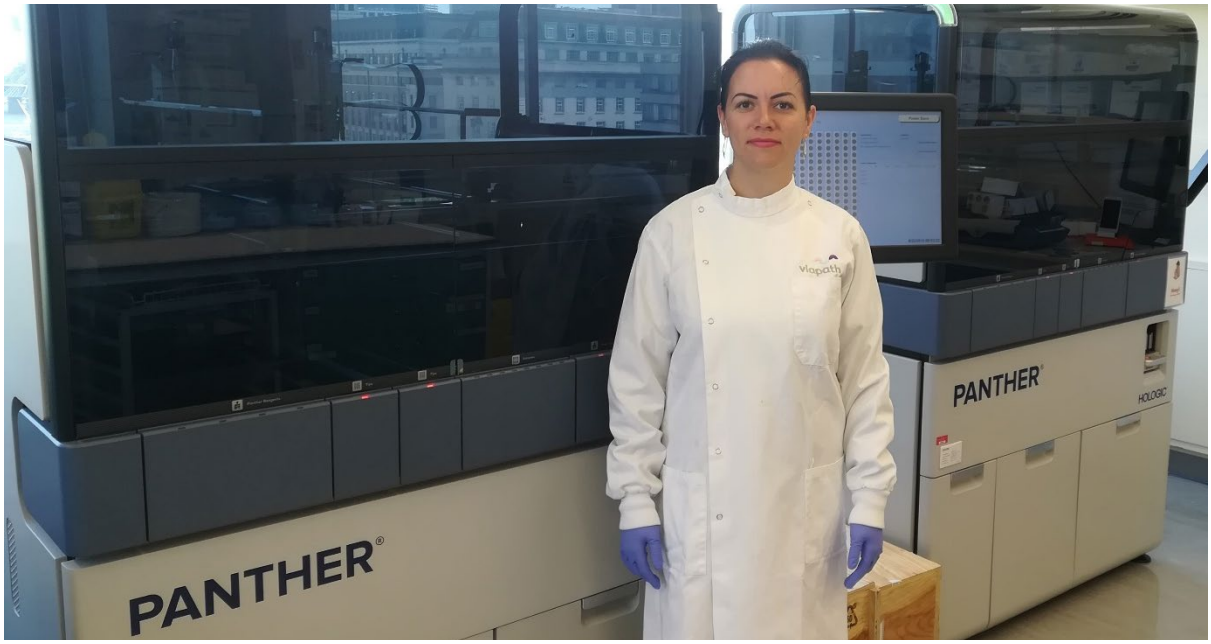


Figure 2: Cristina Vidican, MLA Virology, Infection Sciences, St Thomas' Hospital with Hologic Panthers

Overall, the Aptima assay performed well with slightly higher sensitivity than the real-time PCR assays used by other laboratories.

What can be expected from the new service?

The Aptima *M. genitalium* assay is suitable for vaginal swabs (clinician and self-taken), endocervical swabs, urethral swabs, and first-void urine samples. As part of our verification, rectal swabs and "3in1" (a rectal swab, throat swab and urine combined) samples were also validated, demonstrating high sensitivity with these sample types making the assay an attractive option for *M. genitalium* testing.

And in the future?

The assay detects the presence of *M. genitalium*, but does not provide information on the presence of gene mutations causing antibiotic resistance. Further work is therefore planned to validate another assay for detection of *M. genitalium* antibiotic resistance. Watch this space for further information as it becomes available.

Where can you find out more?

Viapath website - http://www.viapath.co.uk/tests-index

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Virology Enquiries (viroenquiry@gstt.nhs.uk)

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Testing for Sezary Syndrome by Flow Cytometry

Figure 1: Oliver Galvez testing for Sezary by flow Cytometry

What is Sezary Syndrome?

Sezary Syndrome is a rare condition which falls into a group of cancers known as cutaneous T-cell lymphomas. These cancers characteristically affect the skin causing lesions. Sezary Syndrome is an aggressive form where cancerous T lymphocytes, known as sezary cells, are present in the blood, skin and lymph nodes. Morphologically, these sezary cells have an abnormally shaped nucleus described as "cerebriform". This feature, along with the high nucleus-to-cytoplasm ratio, deep and narrow nuclear indentations, condensed chromatin at the nuclear membrane and a cytoplasm depleted of organelles, form the basis of the manual blood film assessment to identify and quantify these cells. However, this is a subjective process as it can be difficult to distinguish sezary cells from other small lymphocytes.

The New Test

Viapath's Special Haematology Laboratory at Guy's Hospital has been working with Dermatology to introduce a new flow cytometry test for patients with Sezary Syndrome. The immunophenotyping protocol to identify and quantify sezary cells is based on detecting the presence and absence of antigens on the lymphocyte cell surface allowing for a more accurate assessment.

Testing by flow cytometry is now a requirement for disease classification and staging. It is also necessary for enrolment and monitoring in clinical trials, important in a disease which currently has a relatively poor prognosis.

The International Society for Cutaneous Lymphomas (ISCL) proposed the following for diagnosis: a Sezary cell count ≥1000/μL, CD4/CD8 ratio ≥10, an increase in circulating T cells with aberrant marker expression (loss of T-cell markers CD2, CD3, CD4 and /or CD5), and evidence of a T-cell clone in the peripheral blood. In addition, according to the ISCL/EORTC classification, a loss of CD7 and CD26 expression by sezary cells and suggested CD4+CD7−cells of at least 40% and CD4+CD26− cells of 30% needs to be determined for a stage classification as a B2 (High blood-tumor burden) if the morphology assessment is unable to distinguish the sezary cells.

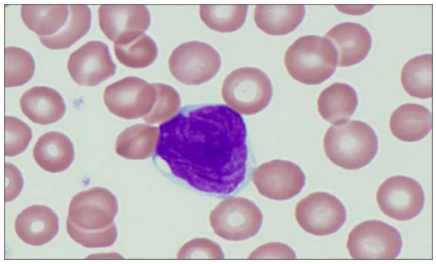

Figure 2: Sezary cell showing the characteristics morphologyc feature, shaped nucleus described as "cerebriform"

Our new test is designed to analyse anomalies in the normal phenotype of T cells, the ratio of CD4 / CD8, as well as the loss of CD7 and CD26. In turn, it provides the percentage of cells as well as their absolute value.

Future work

In February 2018, the 1st EORTC and Euroflow meeting for Sezary Syndrome reviewed the disease and existing practices for the identification of sezary cells. Now the aim is to establish a working group to standardise classification and analysis of the disorder, as has been done with other flow cytometry analyses. Funding is also being sort to further this work, with but also to widen the remit to other aspects such as the investigation and identification of better biomarkers for sezary cells.

For further information, please contact

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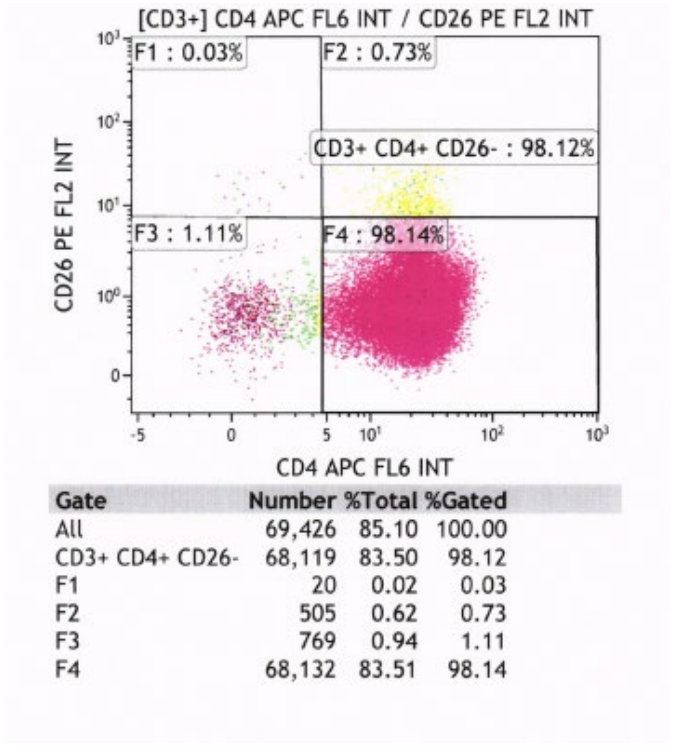
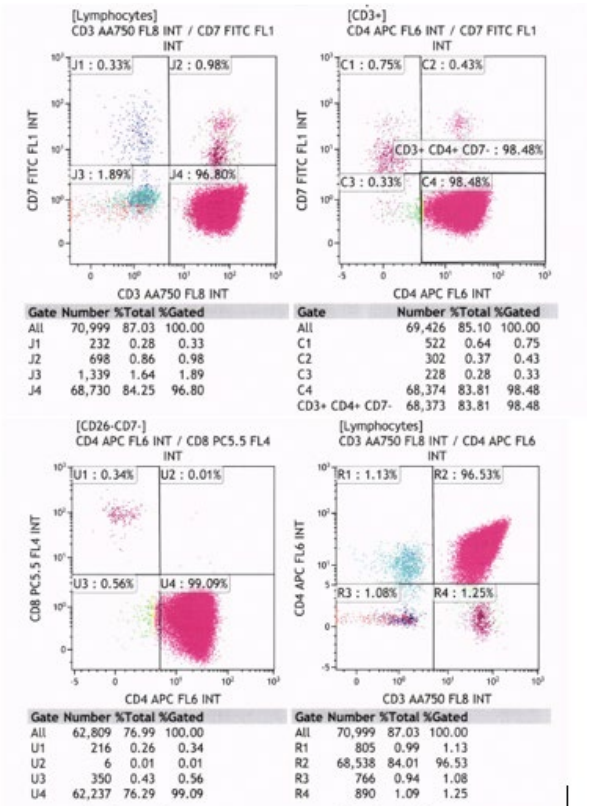
Figure 3: Plots from the sezary panel used in Viapath special haematology laboratory based at Guys Hospital



Gate	Number	%Total	%Gated
All	69,426	85.10	100.00
CD3+ CD4+ CD26-	68,119	83.50	98.12
F1	20	0.02	0.03
F2	505	0.62	0.73
F3	769	0.94	1.11
F4	68,132	83.51	98.14



Gate	Number	%Total	%Gated
All	70,999	87.03	100.00
J1	232	0.28	0.33
J2	698	0.86	0.98
J3	1,339	1.64	1.89
J4	68,730	84.25	96.80

Gate	Number	%Total	%Gated
All	69,426	85.10	100.00
C1	522	0.64	0.75
C2	302	0.37	0.43
C3	228	0.28	0.33
C4	68,374	83.81	98.48
CD3+ CD4+ CD7-	68,373	83.81	98.48

Gate	Number	%Total	%Gated
All	62,809	76.99	100.00
U1	216	0.26	0.34
U2	6	0.01	0.01
U3	350	0.43	0.56
U4	62,237	76.29	99.09

Gate	Number	%Total	%Gated
All	70,999	87.03	100.00
R1	805	0.99	1.13
R2	68,538	84.01	96.53
R3	766	0.94	1.08
R4	890	1.09	1.25

MRI virtual reality application helps children overcome their fears

The 2018 Viapath award for Innovation in Healthcare Science has been awarded to Jonathan Ashmore and Cormac McGrath, from NHS Highlands and Belfast Health and Social Care Trust, at the Advancing Healthcare Awards ceremony for their work helping paediatric patients to prepare for an MRI scan.

Children requiring an MRI scan can feel scared and anxious and the benefits of preparing children for an MRI are well known. Studies have shown that around 47% of children require a general anaesthetic for their scan, a figure that is reduced to 27% if appropriate preparation is used. This fact prompted the hospital play specialist team to approach the MRI department to obtain photographs of the scanner to use in preparing paediatric patients. Coincidently, at the same time, Jonathan had been "playing" with a 360 camera, trying to capture footage from within the scanner bore with the idea of using it for patient preparation. The play specialist team jumped at the chance of having this resource and so the project of using the 360 video to create a full virtual reality MRI experience began.

A team of physicists, play specialists, radiographers and an app developer came together to develop the app, which, when used with a virtual reality (VR) headset and a standard mobile phone, allows children to feel as though they are inside an MRI scanner and experience what it will be like on the day of their scan.

To accompany the app, an MRI preparation book was also developed showing the full MRI journey, with clickable links to load the 360 videos from YouTube, which could also be displayed in a virtual reality headset.

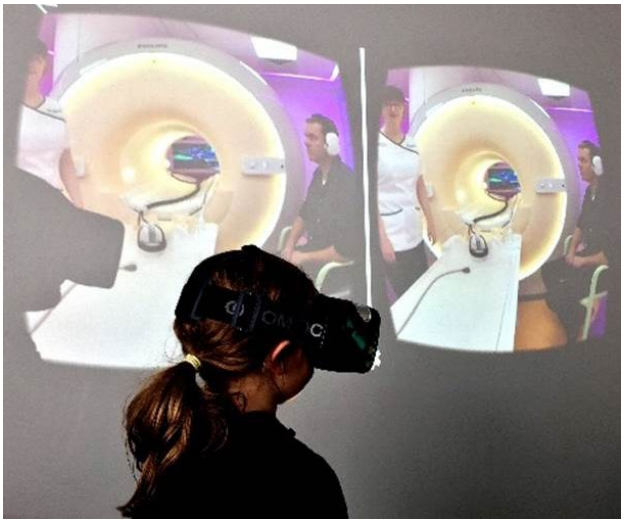


Figure 1: The app with a VR headset in action, demonstrating the equipment and what the child sees

The app has been extremely well received. Unanimously, it was thought to have had a positive impact, and children seemed to find it enjoyable, informative and genuinely helpful to relieve their anxieties. One child even exclaimed "I can't wait for my MRI scan!" Even though the app is targeted at children, an unexpected outcome was the impact on parents who said the app made them feel much less anxious about their child's scan. It also reduced the need for some patients to be anaesthetised during the scanning, which had the added benefit of both reducing cost and increasing patient throughput.

Figure 2: An example of material given to a child prior to their scan

Harvey's Gang visits the Inherited Metabolic Diseases Laboratory

Harvey's Gang is an initiative, which allows young patients with lifelong or sometimes terminal conditions to become trainee scientists for a day, and tour pathology laboratories with their families. Each tour is different and tailored to the child's need and is designed to give the young patients and their families more knowledge about the laboratory aspects of their treatments. The initiative started in the Hematology and Blood Transfusion Laboratory at Worthing Hospital and was named after a young patient who died of leukaemia.

On Wednesday 11th April, Aimee a 9 year old girl with phenylketonuria (PKU) visited the Inherited Metabolic Diseases laboratory at St Thomas' Hospital, as the laboratory analyses her blood spot samples fortnightly. Aimee was chosen by her dieticians as a great person to start the Harvey's Gang adventure. She was already a keen scientist before visiting the laboratory, with science kits and crystal-growing experiments already common activities at home. The week before the visit, Aimee had dressed up as a scientist for her school's fancy dress day with the theme 'What I want to be when I'm older'.

The visit started with a tour of the laboratory and the staff were able to explain how they analyse Aimee's blood spot samples. Aimee was also able to visit the new-born screening laboratory as this is the laboratory that screened Aimee when she was just five days old, enabling the diagnosis of PKU to be made and treatment to be started very quickly.

Aimee carried out some chromatography experiments using felt tip pens and filter paper, and another separation experiment using oil, soap, maple syrup and coloured water to represent the components of blood.

Aimee also had a chance to punch some food colouring spots using a hand puncher and blood spot punching machine (her favourite activity). Other activities included word searches, laboratory I-spy and colour-in a lab coat, all of which Aimee loved, and a highlight was receiving her very own Harvey's Gang lab coat.

The Harvey's Gang initiative is also a great day for the parents and carers of the patient to ask the laboratory staff any questions they may have. Aimee's mother was pleased to meet the scientists who process Aimee's samples and was happy to gain more insight into how the results are generated.

The initiative also gives the laboratory staff an opportunity to meet some of the patients whose samples regularly come into the laboratory, as this makes analysing the results more personal for the staff. The IMD laboratory analyses about 120 blood spot samples from patients with PKU every week, so staff become familiar with patient names but never get to meet the patients.

Many thanks to Erin Emmett and Louise James for putting the visit together and the IMD Team for their support.

"We become familiar with patient names, but it was a real privilege to meet Aimee. It makes analysing the samples more personal"

-

Erin Emmett, Principal Clinical Scientist

Figure 1: Aimee and mum, Zoe, meet some of the scientists who analyse her samples every week.

Figure 2: Aimee mixes together 'red blood cells' (maple syrup), 'white blood cells' (soap), 'plasma' (red water) and 'platelets' (vegetable oil).

Haematoxylin – the story of the blues

The farther back you can look, the farther forward you are likely to see - Winston Churchill

The history of the blues

Haematoxylin is the most well-known dye used in pathology. It remains the primary technique for the demonstration of microscopic nuclear details of cellular and tissue components. However, the story of this remarkable dye dates back hundreds of years, encompassing many different applications and stretching far beyond the histological study of cells and tissues.

Haematoxylin, derived from the Greek words for blood (hematos) and tree (xylos), was originally obtained from the tree Haematoxylon campechianum, found in the Yucatan Peninsula, Mexico[1-3] and documented by Spanish explorers who landed in Campeche in 1517. However, long before this, the Mayan civilisation used extracts from these trees for a multitude of applications, such as a dye to colour cotton-based fabric and a treatment for diarrhoea[1-2].

Figure 1: Photograph kindly donated from Ms Judy Brincat, showing the Haematoxylon Campechianumlogwood with the delicate yellow/ white flowers characteristic of the tree.

Since dyed fabrics were novel in Europe, most common clothing in the fifteenth century was rather drab so interest rapidly grew in the use of the logwood extract as a fabric dye. Its trade became largely dominated by the Spanish and was increasingly profitable, making them the envy of European society. At its height, weight for weight, the logwood was more precious than nearly all other merchandise, with an average load being worth more than a year's cargo of any other commodity[4]. As such, the Spanish galleons exporting logwood back to Spain became the focus of pirates and Buccaneer's alike, including Sir Walter Raleigh, who was known to hide his ship off the Spanish Azores, to then swoop on the Spanish ships and steal the much sort after logwood. Portuguese explorers were also harvesting another dye wood from Central and Southern America, called brazilin. This red heartwood resembled the glowing embers or coals of a fire and, in Portuguese, the word to describe this is 'brasa'. Hence from this came the country's name, Brazil[2].

Figure 2: Photograph of a cross section of Haematoxylon campechianum logwood showing the rich vibrant colour of the wood used to extract haematin.

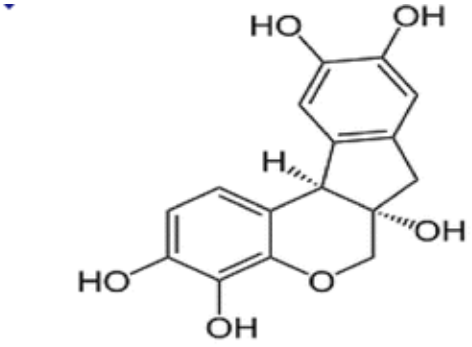
The Dutch and French also expressed interest in acquiring the Haematoxylin logwood. However, the Spanish claimed a monopoly on all logwood sales and to the profits from established logwood plantations, which led to the seven years' war between the Spanish, French and English (1756–63). The English, during this time, discovered the Haematoxylin Campechianum trees growing in British Honduras (Belize). Fights over the rights of British to settle and cut these, led to the Treaty of Paris (1763) which gave rights to the British to cut logwood but sovereignty to the Spanish. The British employed woodcutters called 'Baymen' and, such was their notoriety, the national flag and currency of Belize depicts the Baymen[4].

Over the centuries, haematoxylin continued to find a use as a fabric dye and was used to stain the uniforms of both the North and South American soldiers during the American Civil War (1861–65). Similarly, it enjoyed another renaissance during both World Wars[1-2], when access to the German manufactured, alternative synthetic dyes, was prohibitive.

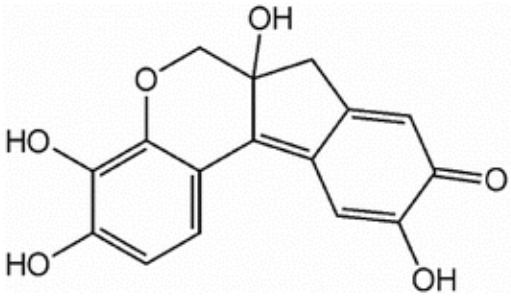
During the Elizabethan era, early fabric dyers in England found the colours of haematoxylin to lack permanency. This paved the way for the introduction mordant, which imparted a long-lasting permanency to the dye. Haematoxylin was extracted and oxidised in boiling water to form hematein. Hematein is a complex phenolic compound similar to flavonoid pigments of flowers. There are two basic procedures which convert the haematoxylin to hematein, natural oxidation by exposure to light and air or chemical oxidation employing either sodium iodate or mercuric oxide and potassium permanganate. The chemical method is much faster and results in instantaneous oxidation.

Haematoxylin is a dark blue or violet stain which is basic and positively charged and will bind to substances such as DNA/RNA. The premise of the interaction is that both DNA and RNA in the nucleus and RNA in ribosomes are acidic due to the presence of the phosphate backbones which are integral to the composition of nucleic acids and which are negatively charged. The negatively charged backbones subsequently form salts with basic dyes containing positive charges. Therefore haematoxylin will bind to DNA and RNA and stain them violet.

Hematein is anionic with poor affinity for tissue. It requires the presence of a mordant to impart a positive charge to the complex thus enabling binding to anionic tissue components like nuclear chromatin. The word mordant is derived from the Latin word mordere which is meaning 'to bite'. Mordants are derived from heavy metals such as aluminium, iron, lead, tungsten and molybdenum[1-2]. They are di/tri valent salts or hydroxides of metals which combine as hydroxides with the dye by displacing a hydrogen atom from the dye (Figure 3). The remaining valences of the mordant serve to attach/bind the dye–mordant complex to the tissue components such as phosphate groups to nucleic acids. The result is a more permanent dye colour[5].



The molecular structure of haematoxylin prior to 'ripening', via oxidization by natural air and sunlight with boiling water or chemically using either sodium iodate or mercuric oxide and potassium permanganate



The molecular structure of hematein following the oxidization of the haematoxylin molecule.

Figure 3: Schematic drawing of the molecular structure of haematoxylin and its subsequent oxidation to form hematein.

The use of the blues in pathology

The application of a mordant to the oxidised haematin dye paved the way for the application of haematoxylin in pathology. It is generally accepted that Waldemeyer introduced the use of haematoxylin in histological examinations in 1862[5]. However, debate continues with references to Reichel's work in 1758[6], using a simple unmordanted solution to study plant material. Bohmer[7] introduced the use of an alum mordant in combination with haematoxylin to obtain selective staining in 1864. Other great landmarks included the introduction of the iron, alum haematoxylin, by Heidenhain in 1892[8] who overcame its instability by adding glacial acetic acid and produced his formula for haematoxylin, still used to this day.

Hematein forms a complex or 'lake' with the metal iron molecules but the nature of these complexes is still not fully understood. The word 'lake' is derived from 'Lacca'. Kerria Lacca is an insect found in India and Thailand, which secretes a deposit on trees. This is dissolved in ethanol to make Shellac, popularly used as French polish and in nail varnish (chip free). Over time the term lacca or 'lac' has changed to lake and now this is generic term for all dye–mordant complexes[9].

Haematoxylins are classified according to the mordant they contain and there are now many types of haematoxylins available, with differing staining qualities for differing tissue types, nearly all named after those who created them. Examples include aluminium-based mordant haematoxylins such as Ehrlich's, Mayer's, Harris', Gill's, Delafield's, Cole's and Carazzi's; iron-based mordant haematoxylins such as Weigert's, Heidenhain's, Verhoeff's and Loyez's; tungsten-based mordant haematoxylins such as phosphotungstic acid haematoxylin (PTAH); molybdenum-based mordant haematoxylins such as Thomas' and lead-based mordant haematoxylins such as Mallory's.

Haematoxylin remains the most popular used dye in histology and one of the most influential dyes of all time. However advances in modern day histopathological interpretation have seen the rise of molecular methodology and its influence on the replacement of traditional micro-anatomical histological and cytological studies. Yet it is quite clear that pathological interpretation hinges on the subtleties of the interpretation of the haematoxylin combined with eosin stain (H&E) and H&E staining has remained a gold standard and 'go to stain' for pathologists for many years. It has been adapted for use on automated platforms, massively increasing productivity and quality, and, perhaps more importantly, have enabled the standardisation of staining protocols for routine diagnostic use (Figure 4)[11]. The role of dye substitutes such as Tango, Newly Blue and Phoenix Blue remains unclear[10] as none have managed to replace haematoxylin despite the shortages of its supply in the twenty-first century. It is extraordinary that the haematoxylin-eosin stain has stood the test of time as the standard stain for histologic examination. Furthermore this simple and inexpensive dye combination can produce enormous amounts of information about the cells functions or aberrations; yielding many diagnostic clues that might otherwise have been missed[12]

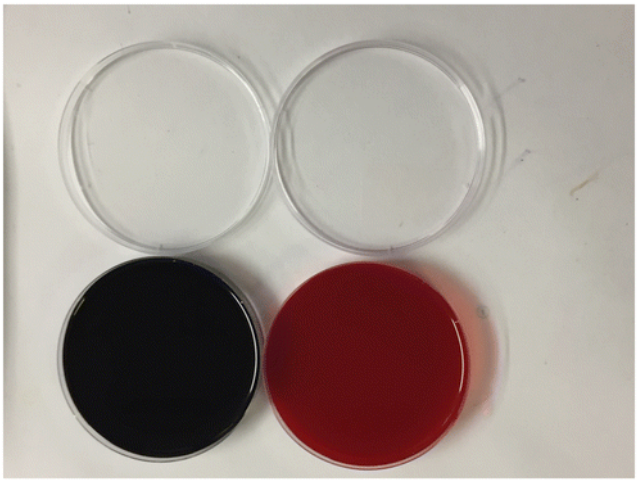


Figure 4: Two petri dishes one containing Harris's haematoxylin (left) and eosin Y (right).

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A Conversation with Viapath's CEO Dougie Dryburgh

Earlier this month Aimee Rhodes, one of Viapath's Future Leaders, interviewed Viapath's Chief Executive Officer about his career and the future direction of Viapath.

Listen to the full interview by following the link: https://www.mixcloud.com/ViapathFutureLeaders/a-conversation-with-dougie-dryburgh-ceo-of-viapath/

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