Issue 6: June 2017

pathology@viapath

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Message from the Editor

In recent weeks, Bob Dylan gave his Nobel Laureate speech and this edition of *pathology@viapath* reminds me of his song *The times they are a changing*. The world around us is in a state of flux and the realm of pathology is no exception. There is a revolution taking place in genetics, screening to prevent cervical cancer is advancing, steroid profiling is evolving and new tests are being introduced. These articles help to illustrate how pathology is moving forward; I hope you find them interesting.

Viapath is involved with many other aspects of pathology, so if there are any subjects that you would like to be covered in future editions of *pathology@viapath*, please get in touch!

Cervical cancer screening in England — The journey so far

Cervical cancer prevention has been based on cervical cytology screening for the last 50 years and has been a major success in the UK. With recent rapid advances in understanding of human papillomavirus (HPV), its role in carcinogenesis, the clinical applications of primary prevention by HPV immunisation and secondary prevention by HR-HPV testing, the approach to cervical cancer prevention in the UK is undergoing significant change.

In the beginning…….

Screening for cervical cancer has played a significant role in medicine since the late 19th century, when public health authorities identified the need to detect specific diseases in certain groups of the population. However, it is only relatively recently that screening for the early detection of cancer has gained widespread acceptance. Fundamental to this was the pioneering work in the development of techniques of exfoliative cytology initiated by Dr George Papanicolaou who, in 1941, described how this test could be of value in the early detection of cervical cancer. He went onto describe techniques to sample, process and stain cells to identify precancerous changes and became known as "The Father of Cytology".

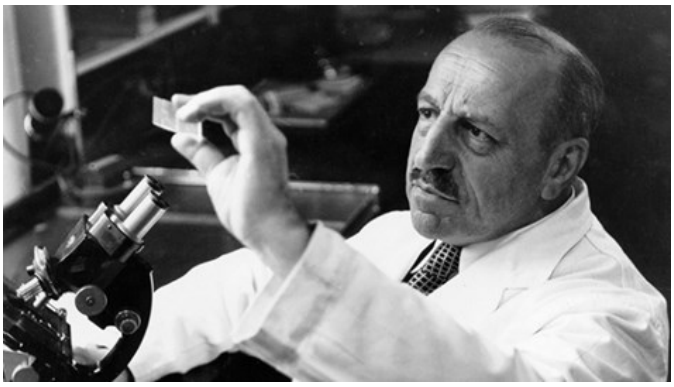

Figure 1: Dr Papanicolaou

The "Pap" test (or "Pap" smear), named after Dr Papanicolaou, had all the criteria of a screening technique for cancer, as described by Cochrane and Holland in 1971 who recommended:

- Simplicity – The sample should be easy to take.
- Acceptability – The method of sampling must be acceptable to the population involved.
- Accuracy – The test results must give a true measurement of the disease under investigation.
- Cost – The expense of screening must be considered in relation to benefits of early detection of disease.
- Precision – The tests must give consistent results in repeated trials.
- Sensitivity – The test should be able to detect all individuals with the disease.
- Specificity – The test should not identify positive results in non-diseased individuals.

Early screening in England

In 1964, cervical screening was introduced in England but it was quite disorganised. Cervical "smears" were taken from many women, most opportunistically, but abnormal results were infrequently followed-up. Policies regarding which and how women should be screened were inadequate. Harsh treatment regimes coupled with no classification system for cellular changes increased the risk of over-diagnosis and overtreatment.

Mortality rates from cervical cancer in women <35 years old rose alarmingly leading to increased awareness of the limitations of cervical screening. By the mid 1980's a rise in cervical abnormalities resulted in backlogs which created further public dissatisfaction with the cervical screening service. Reports emerged of organized, public-health centred screening programmes using a population-based registry to recall women which had successfully reduced mortality.

The birth of the NHS cervical screening programme (NHSCSP)

In 1988 a centralised NHS cervical screening programme was launched. Pivotal to this was a centrally agreed policy regarding who to screen and treat. The Department of Health (DOH) requested district health authorities to implement a computerised call/recall system. Women aged 20-64 years were invited to participate in cervical cancer screening every 3-5 years, leading to a significant increase in the number of women tested.

However, inadequate samples comprised 7-9% of total tests, requiring tests to be repeated and 3 consecutive inadequate test results required referral to colposcopy (a simple procedure used to look at the cervix), due to the increased risk of undetected cancerous precursors. 5-10% of tests were categorised as borderline changes of mild precancerous changes (dyskaryosis), 1% had moderate dyskaryosis, and 0.5% had severe dyskaryosis. Treatment options for moderate and severe dyskaryosis were clear. However, treatment options for borderline changes and mild dyskaryosis ranged from cytological surveillance to referral for colposcopy.

A series of errors within the cervical screening programme identified the need for external quality assurance and proficiency testing. One widely publicised failure occurred at Kent and Canterbury Hospitals NHS Trust; inadequate training of screening staff and poor supervision had been raised. These concerns were acknowledged, which led to 90,000 tests being re-examined. A public enquiry followed which recommended the reinforcement of quality assurance protocols within the NHSCSP. Responsibility for screening was placed with the directors of public health, which prompted the government to implement a mandatory accreditation system for all cervical screening laboratories in the UK.

Advances in technology drive change

During 2000-2010, the advent of Liquid-Based Cytology (LBC) (which involves making a suspension of cells from a sample and producing a thin layer of cells on a slide) presented opportunities to evaluate the role of high risk HPV (HR-HPV) testing as well as the potential to automate the screening process. Tests introduced into the NHSCSP are subject to rigorous evaluation and this suggested that LBC may reduce inadequate rates, improve sensitivity and reduce the time taken to screen a sample. Three pilot sites were set up and run from 2001-2002 in England, with the conclusion that LBC had comparable sensitivity and specificity to conventional cytology, but led to far fewer inadequate results, requiring fewer repeat tests and was cost-effective. National rollout was implemented in 2003 after endorsement by NICE (National Institute of Clinical Excellence) and completed in 2008.

LBC provided a means for additional reflex testing for HR-HPV which facilitated triage of women with low grade abnormalities who were at greater risk of developing cervical cancer and hence required referral to colposcopy. Triage of low grade abnormalities via HR-HPV testing reduced the number of repeat tests, however increased the number of referrals to colposcopy. A refined protocol was used for a national rollout, which included an HR-HPV as a test of cure (TOC) for post treatment follow-up to identify HR-HPV negative women who can be safety returned to routine recall, instead of annual cytology follow-up for 10 years. This utilises the high negative predictive value of HR-HPV testing to identify women of rare risk of CIN2 or worse. In 2011, Advisory Committee for cervical screening recommended to the government that HR-HPV test for triage and TOC be implemented as this leads to a "more patient-centred service and major cost saving". Rollout concluded in 2014.

In 2003, based on evidence suggesting that cervical screening was not as effective in women under 25 years old, a key policy change was to raise the age at which screening commences to 25. This was reviewed by the Advisory Committee in 2009, due to increased public pressure to reduce the age to 20 following the publicity surrounding the death of Jade Goody from cervical cancer. However, evidence showing that screening had little or no benefit to women under 25 years old, resulted in the age not being lowered.

In 2003, the screening frequency for routine recall was increased for women aged 50-64 to 5 yearly, based on an audit which concluded that 5 yearly screening provided considerable protection for this age group.

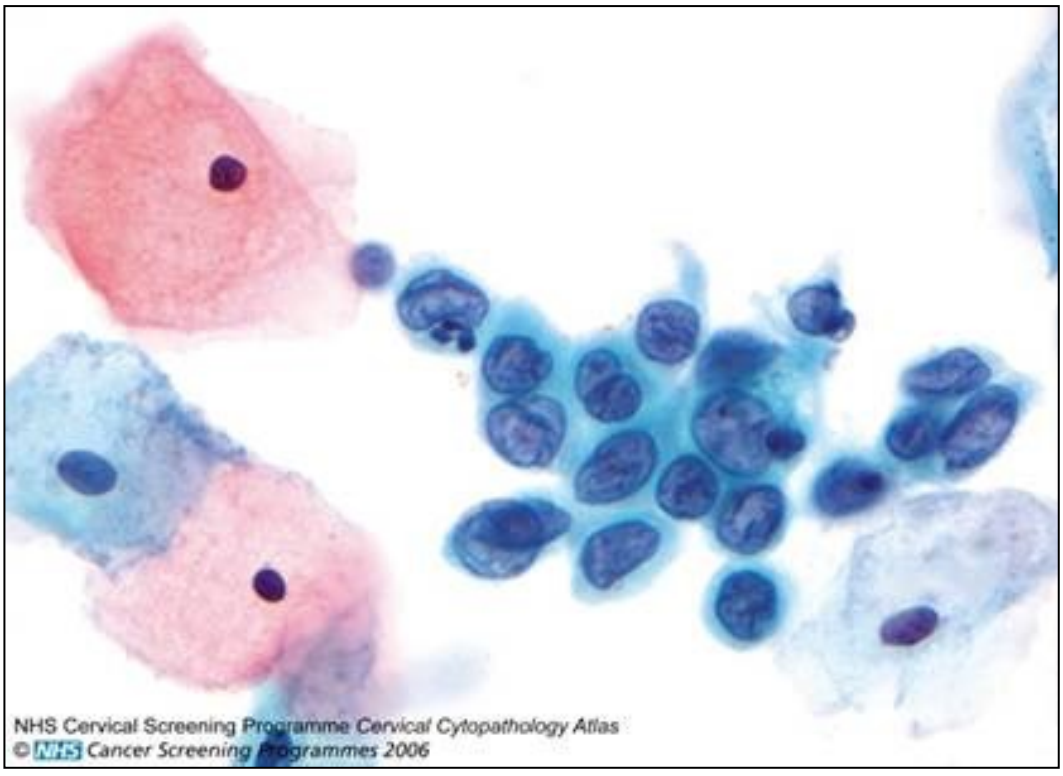

Figure 2: High grade (severe) dyskaryosis

Figure 3: St Thomas' Hospital Cervical Cytology team

In 2007, the Cancer Reform Strategy announced that all women should receive their cervical screening tests results within 2 weeks of sampling and that this would be implemented by 2010. This metric was added as a vital sign of the operating framework for the NHS in England 2010/11. Laboratory re-configuration was recommended to produce larger more efficient laboratories alongside larger call/recall centres which was backed by Lord Carter of Coles' independent review of NHS pathology services in 2016.

Cervical cancer prevention has been based on cervical cytology screening and has been a major success

Where do we go now?

HR-HPV primary screening had been tested in the HPV pilot sites since HPV triage and TOC rollout. In 2016, the UK National screening committee recommended that HPV primary screening should be adopted by the NHSCSP and an announcement was made that HPV primary testing would be implemented in England by 2019.

Primary HR-HPV screening is a complex pathway which will use the HR-HPV test as the primary test. Only tests with a positive HR-HPV result will have a slide produced for reflex cervical screening, reducing the current volume of cervical cytology work by approximately 87%.

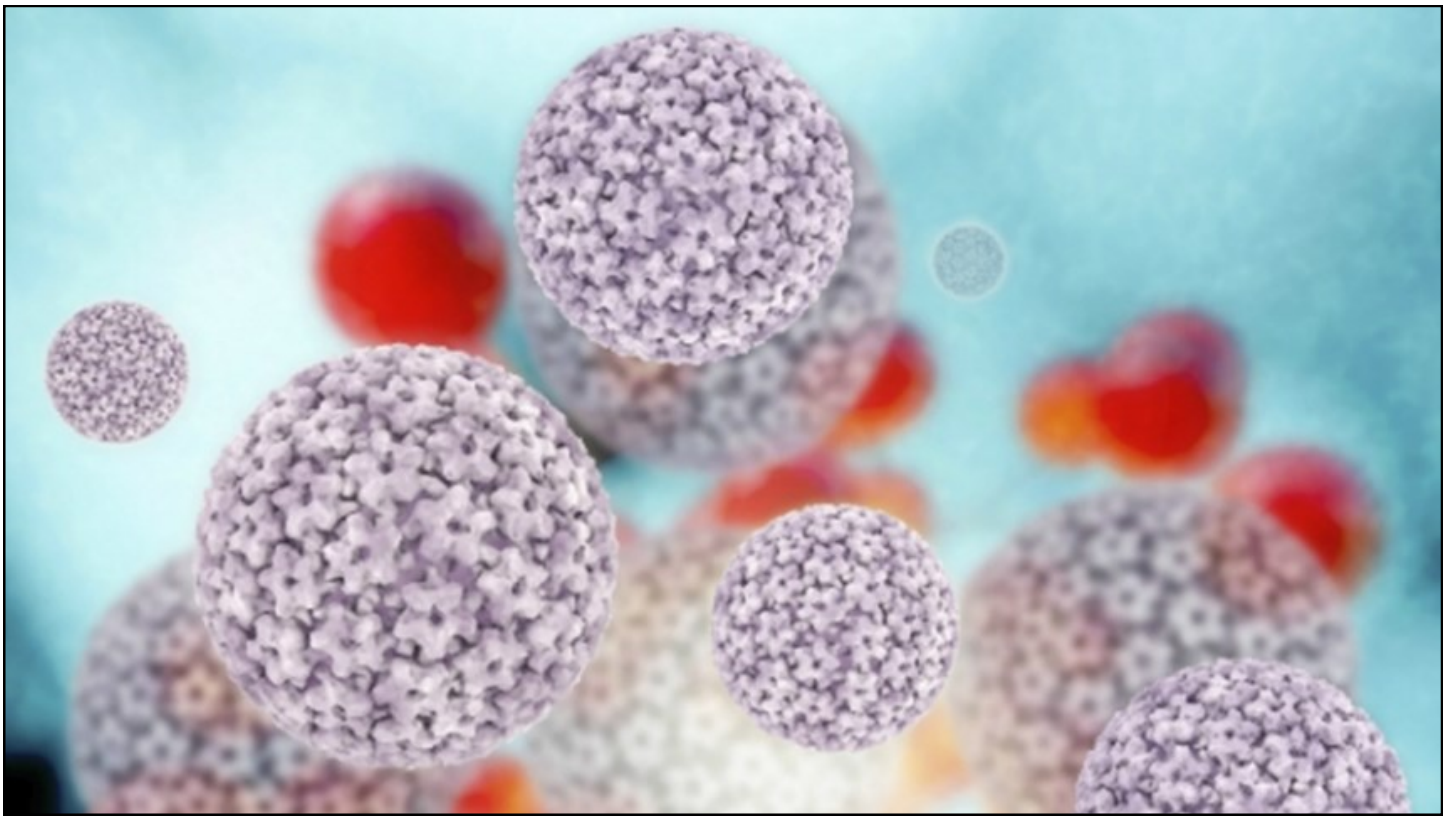

Figure 4: Human papillomavirus (HPV)

The HPV primary screening implementation group recommended, in 2017, that there should be 5-15 cytology screening centres linked to HPV test centres throughout the UK. These future centres will require robust IT systems to support them. Staff training and future workforce requirements are being carefully considered in light of the impact of primary screening on the workforce, with staff retention being a significant concern. Now NHS England, who commission the NHSCSP, is working through how this preferred service model can be implemented by 2019.

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References:

M. Bibbo. Comprehensive Cytology. *W.B. Saunders Company:* 2nd edition, 1997.

R. Albrow, H. Kitchener, N. Gupta, M. Desai. Cervical screening in England: The past, present and future. *Cancer Cytopathology.*120: 87-96 2012.

R. Stubbs. HPV primary screening in the cervical screening programme. *Public Health England:* April 2016.

R. Stubbs. Deciding how best to roll out HPV testing as the primary cervical screening test. *Public Health England:* January 2017.

T. Freeman-Wang, M.E. Cruickshank, H.C.Kitchener. Progress in Cervical Screening in the UK. *Royal College of Obstetricians and Gynaecologists*. Scientific impact paper 7, March 2016.

Cancer Research UK. HPV and Cancer. www.cancerresearchuk.org/about-cancer/causes-of-cancer/infections-hpv-and-cancer/hpv-and-cancer, March 2017.

New assays: For Pregabalin, Gabapentin and Methylphenidate in urine

Offered by the Toxicology Unit to monitor adherence to treatment, and/or detect illicit use of these drugs

Three new assays have been introduced to Viapath's Toxicology Unit at King's College Hospital. They are for identifying pregabalin, gabapentin and methylphenidate in urine and requesting these assays may aid clinicians to monitor adherence to treatment, and/or detect illicit use of these drugs.

Pregabalin and gabapentin are prescription drugs that are used therapeutically for the treatment of epilepsy and neuropathic pain. Pregabalin may be also be used in the treatment of generalised anxiety disorder. In May 2015, Public Health England and NHS England issued advice to prescribers on the risk of misuse of gabapentin and pregabalin and both drugs are abused for their sedative and relaxant effects.

Methylphenidate is a psychostimulant prescribed as a first-line treatment in Attention Deficit Hyperactivity Disorder (ADHD). It undergoes extensive metabolism, with ritalinic acid being the major urinary metabolite. When methylphenidate is taken concurrently with ethanol, the metabolite ethylphenidate may be formed. More recently, ethylphenidate has been marketed as a 'legal high' and is abused for its stimulant effect. The methylphenidate assay detects methylphenidate, ethylphenidate, and ritalinic acid (each reported separately).

Making a test request

A urine sample should ideally be collected into a universal container and can be transported at room temperature. Each of these new assays can be requested on their own or in addition to a urine drug screen.

For further information please contact: Viapath's Toxicology Unit at Kings College Hospital

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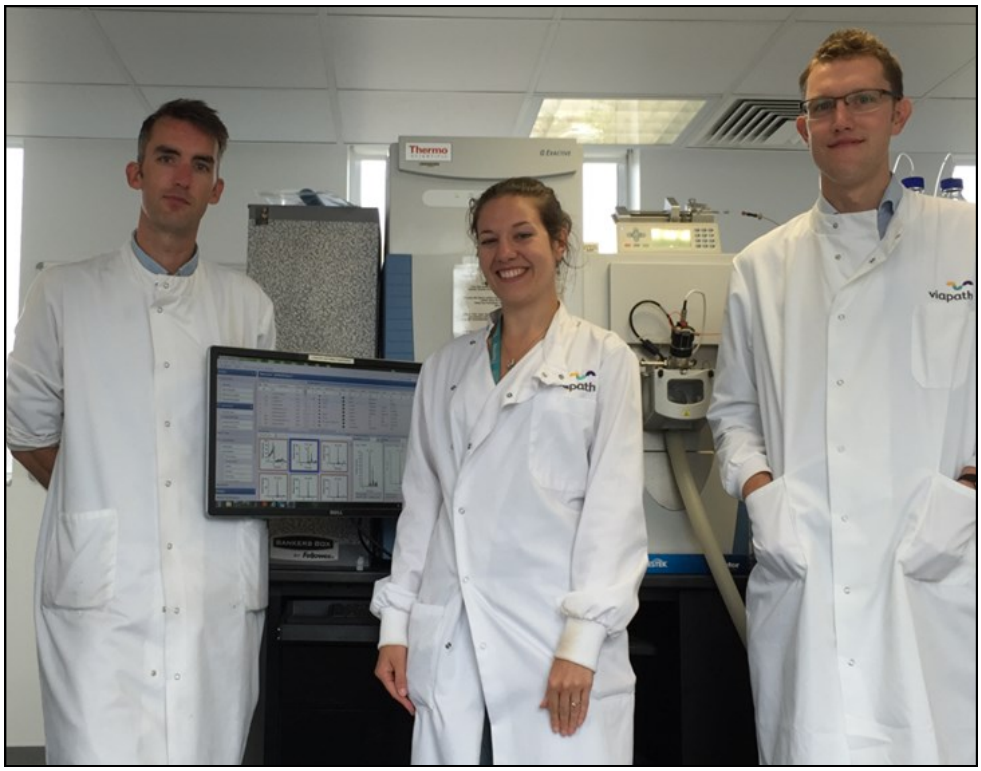
References

Abuse and Misuse of Pregabalin and Gabapentin Kirk E. Evoy, Megan D. Morrison, Stephen R. Saklad. Abuse and Misuse of Pregabalin and Gabapentin. Drugs, March 2017, Volume 77, Issue 4, pp 403–426



The drugs of abuse team and high-resolution mass spectrometer in use for urine testing at KCH

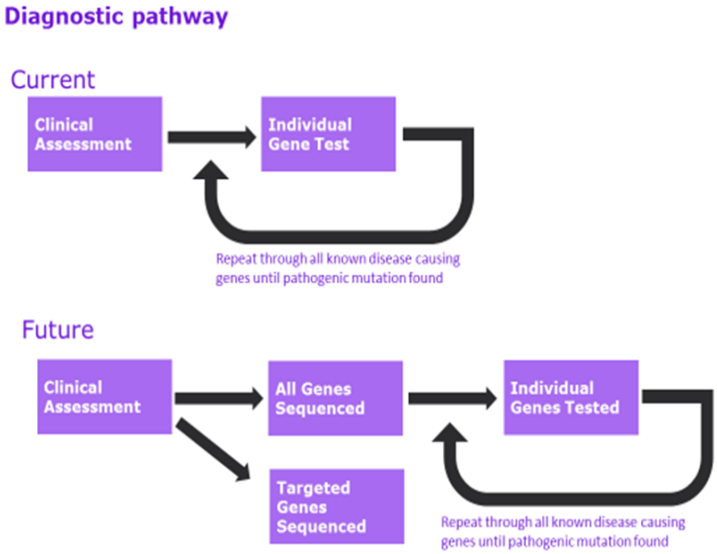
How genetic and molecular innovation in pathology will improve NHS outcomes for patients

The Genetic Revolution

The march of the genetic revolution has seen the understanding of the human genome grow at an exponential rate, surpassing traditional development time lines. Diagnostic and pharmaceutical companies are having to play catch -up with the opportunities and benefits this area of medicine has to offer patients, clinicians and the wider population.

The diagnostic paradigm is set to change, no longer will genetic testing be the final confirmatory test of a proposed diagnostic hypothesis, that has been shaped by countless other traditional pathology tests, but the starting point. It will become the basis of a personalised treatment plan for that individual or even whole families from birth to head off a genetically described future disorder.

The Diagnostic Pathway: Now and then



Current Advances

An example of the advances being made is the work being undertaken by Dr Tony Marinaki, Consultant Clinical Scientist in Viapath's Purine Research Laboratory. Studying microRNAs (miRNA), which are small non-coding RNA molecules that are involved in regulation of gene expression, Dr Marinaki and his team showed in a pilot study that circulating levels of miR-132 were significantly decreased in patients with gestational diabetes mellitus at 24-30 weeks gestation compared to those women without gestational diabetes. These results raise the possibility of an alternative test to an oral glucose tolerance test for the diagnosis of gestational diabetes. The results also build upon the understanding of how gene expression can be linked to circulating markers for predicting disorders.

Personalised Medicine

As well as predicting and diagnosing a pathological disorder, treatment can be targeted. By building an understanding of the genetic make-up of an acquired genetic mutation, such as those seen in cancer, treatments can be personalised to ensure the best possible outcome for the patient, with the clinician being able to target specific monoclonal treatments against ligands, receptors and intracellular signalling components reducing the capacity of the cancer to spread. An example of this can be seen in the use of Herceptin in breast cancer in patients who express the HER2 genotype.

This scientific understanding expands the need for a more comprehensive diagnostic service, it also increases the need to ensure a complete diagnostic picture can be presented for each and every patient enabling best practice and targeted treatment plans that are appropriate, proportionate and with the optimum chance of success.

The anticancer drug 5-fluorouracil (5FU) is widely used for the treatment breast and colorectal cancer but is associated with severe toxicity in about 25% of patients. In work published in the British Journal of Cancer, researchers at Viapath showed that variation in dihydropyrimidine dehydrogenase (DPD), an enzyme involved in the degradation of pyrimidines, predicts severe toxicity to this therapy. On average, patients with a variant human DPD gene (DPYD) genotype spent seven days in hospital due to toxicity. Similarly, data presented at a recent National Cancer Research Conference by breast cancer oncologists from Guy's Hospital showed that in a series of 44 patient, two patients experiencing toxicity were both DPD deficient. This illustrates that using pharmacogenetics to identify and manage at-risk patients will improve their quality of life, clinical outcomes and, even though diagnostic costs rise, the overall costs of patient pathways decrease.

As with all revolutionary steps in healthcare, there are other benefits that are being harnessed. It is not just the human genome that is of interest to healthcare scientists, but those of microbes. The rapid identification of an infective organism can directly improve the outcome for a patient. Basic point of care devices that can detect the DNA or RNA of a microbe are available. These enable the clinical team to target treatment faster, understand outbreaks and cohort patients in wards to reduce hospital acquired infections. Understanding the use and applicability of the technology is the challenge but one which will reap rewards.

Innovation of this branch of pathology is not limited to a better understanding of the genetic basis of disease; it requires diagnostic and pathology providers, healthcare systems, industry and national programmes to build the infrastructure to support this. Much of the genetic revolution has not come from novel techniques, but a better method of handling significant quantities of data, to allow interpretation via clinically robust algorithm pipelines, to adopt and introduce the latest genetic sequencing technology and strategy. This can then be applied to deliver the patient focused approach that will be needed to realise fully the benefits. To put this into context, in recent years referrals for Comparative Genomic Hybridisation (CGH) array testing to Viapath's Genetics laboratory at Guy's Hospital showed that roughly 25% of the results revealed mutations that can lead to intellectual disability. Neurodevelopmental disorders cover a far bigger group than solely intellectual disability and Dr Ahn and his team have been trying to code the phenotype. These findings imply that targeting only one specific gene for neurodevelopmental disorders in the diagnosis of a patient is inefficient and more comprehensive testing strategies are needed to support a diagnosis. To date, Dr Ahn's research has found 1421 genes with reported pathogenic variants associated with a neurodevelopmental phenotype. A collaboration with industry and funding from Viapath's Innovation Academy's Innovation Fund has allowed analysis using next generation sequencing of these genes to further understand the genetic mechanisms by which these conditions occur.

Tony Marinaki and Tamanna Islam

Advances in Technology

Providers are faced with a complex choice of technological approaches each with advantages and disadvantages depending upon the application. The use of HiSeq and Mieq high resolution techniques for high throughput genetic analysis for multiple genetic targets, whole exome and even whole genome, are able to accurately identify mutations in patients with congenital disorders. These are highly productive where the disorder can be characterised by thousands of different mutations to reach a positive diagnosis, but incredibly intensive in terms of the Healthcare Scientist's time as they work through the sub-clinical and naturally occurring mutations. However, when looking for the presence or, indeed, absence of a single mutation or gene, this can be time consuming and costly for a single patient. More targeted technologies may prove to be a better option; such as Ion-Torrent that allows for a "load and go" operation, with all the required algorithms pre-loaded in the instrumentation. This approach is able to pick up a single gene rapidly, giving the clinician the additional information in time for inclusion into the histological report on a biopsy, increasing the support the pathology report has on the advanced management of the cancer patient.

The advances within the field of molecular diagnostics is also allowing the opportunity for new areas of diagnostics, particularly as the understanding of the genome increases the requirement to see the expressed and active molecules in identified mutations. This is giving rise to the clinical applications of epigenetics, proteomics and metabolomics amongst others.

These different technological approaches are costly and fundamentally different. Purchasing either at scale, ensuring a wide panel of instruments, close engagement with diagnostic

instrument providers, guaranteeing the availability of appropriate genetic panels, in the case of Ion-Torrent, or purchasing access to dedicated high throughput sequencing lanes, together with an active development pipeline.

This allows the laboratory to remain responsive to clinical demands for both cancer and constitutional genetics. It is likely that diagnostic providers will need a range of these technologies to deliver the future advances in genetic medicine.

Another consideration that needs to be applied is information management: the transfer and storage of significantly sized files is going to impact upon many healthcare organisations. The size and scale that allows wide scale adoption and integration is on a scale with Radiology Picture Archiving and Communication System (PACS) systems and is likely to be linked into wider digital pathology solutions to enable efficiency of scale.

Adoption at scale across the UK is also limited by the available workforce. Bioinformatics as a separate healthcare science discipline is in its infancy, with the first cohort of nationally funded trainee scientists only part way through their registration training. Experience of existing scientists is concentrated into current large genetics centres, some equipped with strong organisational links that allow the broad adoption of molecular diagnostic techniques with others working in limited silos.

Large pathology providers, especially those that are able to direct their own strategy and investment choices, are well placed to take a national lead in the wide scale adoption of molecular techniques. The co-location of laboratories is not a pre-requisite, in fact allowing for centralisation builds upon the available limited expertise, enabling broader access to this essential knowledge base. Access to a cohort of experts, not just genetic experts, integrated into clinical teams is essential.

It is an often cited fact that technological adoption within the NHS takes on average 14 years. The demand and expectation for pathology departments to deliver a comprehensive molecular diagnostic service across all specialities is prevalent now, pressing even organisations investing heavily in next generation sequencing. In the current economic environment, adoption will come through partnerships with providers that operate at scale, with existing expertise and a proven clinical track record.

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Why urine steroid profiling is still the gold standard for identification of steroid-related disorders

Viapath's Urinary Steroid Profiling (USP) service is one of two Supraregional Assay Service-designated laboratories in the UK tasked with providing steroid metabolite analysis. This is especially useful for the identification of inborn errors of steroid metabolism and steroid-secreting adrenal tumours but can also help other investigations where changes in steroid production or metabolism might be part of the pathology. Steroid metabolite analysis utilises gas -chromatography-mass spectrometry, a long established technique, but one that is still unsurpassed for separation and positive identification of steroids.

Over many years of operation, the laboratory has established the identity of numerous previously unreported steroids in a never-ending quest to refine the usefulness of this approach. By liaising closely with clinicians about individual patients, new insights continue to be gained, such as the recent realisation that sodium loss in babies due to renal and cerebral causes can be differentiated by this technique. Viapath's Urine Steroid Profiling Laboratory is always happy to be consulted about whether this approach might be informative in a current investigation.

Steroid metabolite analysis is especially useful for the identification of inborn errors of steroid metabolism

How urinary steroid profiling can make the difference: A case study

Inborn errors of steroid metabolism may first be suspected in the newborn period. For example, a presentation of ambiguous genitalia might be due to diminished testosterone production in a male or an excess in a female; if they show biochemical evidence of salt wasting, this may be the result of inability to make or respond to aldosterone.

A virilised (masculinised) female baby who developed salt wasting was found to have 3BHSD deficiency

A virilised (masculinised) female baby was born recently. She developed salt wasting. The most common cause of this is 21-hydroxylase deficiency. Much more rarely, the same clinical presentation may be due to 3B-hydroxysteroid dehydrogenase (3BHSD) deficiency. A urine steroid profile rapidly excluded 21-hydroxylase deficiency. A sample collected at 3 months of age, when the steroid biochemistry had sufficiently matured, clinched a diagnosis of 3BHSD deficiency.

Making a test request

For most purposes, an untimed urine sample is sufficient, but when assessment of steroid excretion rates would be informative, as in Cushing's syndrome, a 24 hour collection is preferable. The laboratory will always strive to get a useful result in difficult circumstances such as when only a very small sample can be obtained from a new-born baby. Samples can even be sent in the form of a wet nappy, which may help to avoid an expensive hospital stay.

Since all reports are individually generated, with a detailed comment that aims to answer the specific clinical questions that are raised, it is important to clearly state the reasons for the request and to give information on whether steroids or other drugs are being given. Steroid treatment can in some circumstances make interpretation difficult, so it is important to collect a sample before starting treatment wherever possible. These can be stored in a refrigerator pending a decision on whether the test is needed. The stated turnaround time for full reporting is 21 days, but results can be provided much faster if notice is given.

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Viapath's Urine Steroid Profiling Laboratory:

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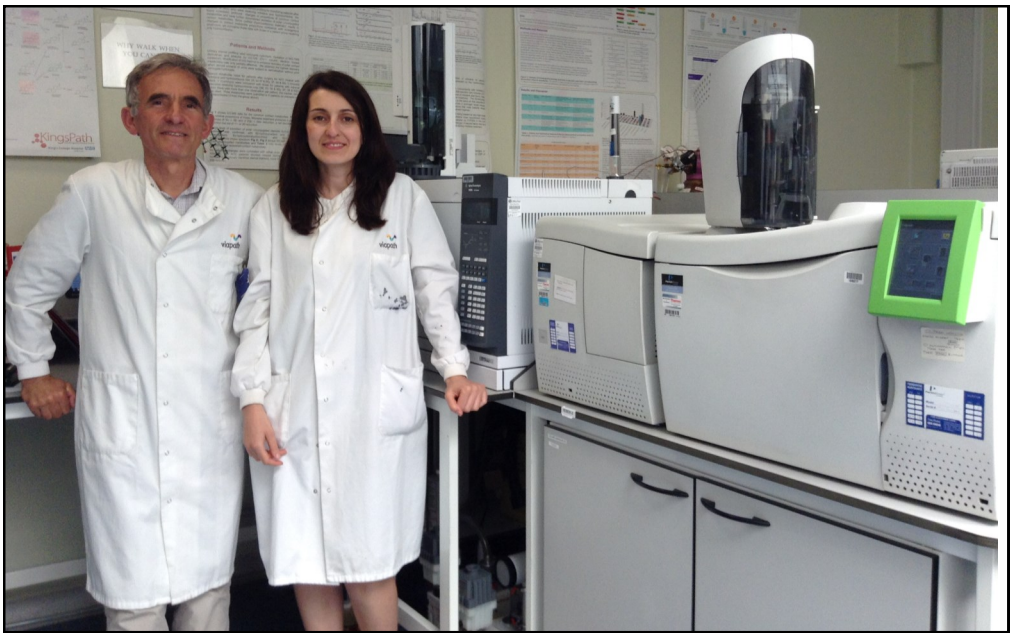
Web:www.viapath.co.uk/our-tests/urine-steroid-profile



Norman Taylor and Lea Ghataore from the Steroid Laboratory

Viapath Career Development Programme

The Future Leaders in Innovation group consists of Viapath employees who are driven to stimulate and encourage positive change within the organisation, and ultimately shape the future of pathology services. One of the innovative new projects that has stemmed from the Future Leaders is the Viapath Career Development Programme (VCDP), the pilot event of which was held on June 9th, 10th 2017. The VCDP was created with the vision of giving Viapath employees, who are at the early stages of their scientific careers, the essential knowledge and skills to achieve their career aspirations. Over the course of two days delegates were given the opportunity to listen to a variety of presentations and participate in numerous practical activities, that not only gave the foundation knowledge and skills required in diagnostic laboratories but also offered a wealth of key transferrable skills. Here are some of the highlights:

The Career Pathway

The inaugural lecture was given by David Wells, Director of Operations for Viapath's Reference Pathology Services, who talked about his inspiring journey from the start of his career to where he is today. David began his career as a Medical Laboratory Assistant in the Haematinics laboratory at Addenbrooke's Hospital and qualified as a state-registered biomedical scientist. This led to more senior and managerial scientist roles, involving setting up a Trace Elements Laboratory from scratch, and ultimately led to his current role at Viapath.

Figure 1: David Wells presenting the inaugural lecture

David's lecture was followed by some short talks about what being a clinical and biomedical scientist entails and how this differs from a genetics career pathway. For instance, in order to qualify as a biomedical or clinical scientist, the Health and Care Professions Council (HCPC) sets certain standards of proficiency that trainees must meet by completing a pre-registration portfolio which is then verified by an external examiner. Moreover, working in laboratories within the genetics department can also involve the role of healthcare science practitioners who have particular expertise in applied scientific techniques used in the diagnosis of disease.

The scientific career pathways talk helped me to consolidate the approach I want to take in my career progression

CV Writing and Interviewing Skills

Next, Rakhee Patel and Paul Sadler, who are part of Viapath's recruitment team, conducted a session on how to wow a potential employer, to stand out amongst the crowd and get that dream job! They gave key advice on how to master the art of writing a good CV and an accompanying personal statement, along with how to win over an employer at the interview stage.

Next Generation Sequencing

'HLA typing – Next Generation Sequencing' followed where Dr Robert Collins, Clinical Scientist and Deputy Director of Viapath's Clinical Transplantation laboratory at Guy's Hospital, explained the role of Human Leucocyte Antigens (HLA) in the immune response and the high level of variation within a population of HLA genes.

How to conquer scientific calculations

David Card, Clinical Scientist in Viapath's Human Nutristasis Unit at St Thomas' Hospital, gave some helpful tips on how to conquer scientific calculations commonly used in the laboratory, such as the use of creatinine clearance, a commonly used marker for renal function and the importance of utilising statistics when evaluating

calibration and quality control data in the nalytical stage of patient sampling.

A Masterclass on Pipetting

Aimee Rhodes, Clinical Scientist in Viapath's Immunology laboratory at King's College Hospital, tested delegates with a series of challenges in a Pipetting Masterclass which focused on the fundamentals of using a pipette correctly. This interactive session highlighted the necessity of both accuracy and precision and involved serial and multichannel pipetting as well as quantifying the accuracy of a pipette.

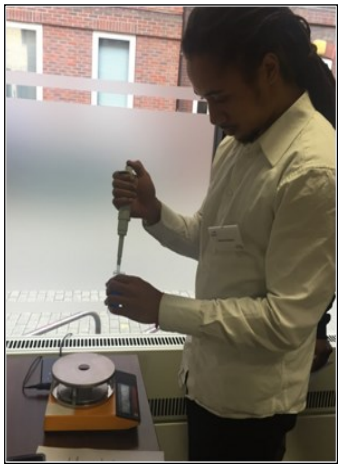


Figure 2a: Measuring accuracy of pipetting using a balance

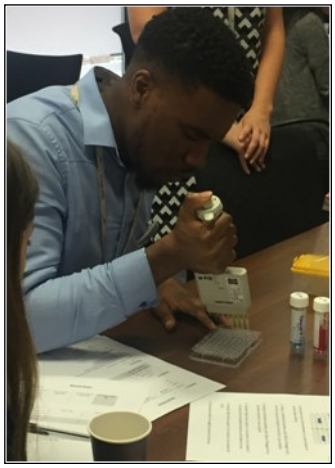


Figure 2b: Multichannel pipetting

A Case Study on Myeloma

Day two kicked off with a fascinating case study on multiple myeloma, presented by Lauren Pitt, Medical Technical Officer in Viapath's Cancer Genetics laboratory at Guy's Hospital, and Aimee Rhodes. The progression of the disease within a patient and the most common presenting features were discussed as well as the repertoire of tests used to diagnose the disease. The heterogeneous nature of this disease requires the test results from several laboratories within different disciplines, such as haematology, biochemistry, genetics and immunology, all of which are required to make a diagnosis and monitor disease progression. This case study will be presented in a future edition of this newsletter.

CPD and Evidence-based Portfolios and Interpreting Manual Techniques

Vikki Woollett-Calnan, Biomedical Scientist in Viapath's Blood Transfusion laboratory at King's College Hospital, gave an incredibly useful presentation about Continuous Professional Development (CPD) and evidence-based portfolios and how these are core aspects of gaining HCPC registration as a clinical or biomedical scientist. She gave examples of the different ways to maintain CPD such as reading journals and articles, working through case studies, attending conferences to name a few. Vikki also emphasised the importance of reflecting on one's practice and implementing new knowledge and skills into every day practice.

Louise James, Biomedical Scientist in the Reference Chemistry laboratory at King's College Hospital, and Vikki Woollett-Calnan outlined the use of manual techniques still utilised in diagnostic laboratories. Examples given included the use of fluid crystals to identify crystalline structures in joint fluid allowing the differentiation between gout and pseudogout; the use of blood films, a vital investigation performed in every haematology laboratory, and their use in the diagnosis and monitoring of haematological diseases such as malaria, haemoglobinopathies and myelodysplastic disorders.

Discover Your Strengths

Finally, Dominic Harrington, Viapath's Chief Scientific Officer, closed the event with some words of motivation for all the attendees such as "discover your strengths and make them your unique selling point" and, most importantly, "be yourself".

The delegates left feeling inspired and more than ready to take on the next steps towards their career. The pilot programme established a strong foundation with the potential for an annual event and being accessible to both external and internal Viapath employees.

Links:

Listen to Pathology Podcasts from Future Leaders in Innovation Group at http://www.viapath.co.uk/news-and-press/pathology-podcasts-from-viapath%E2%80%99s-future-leaders-in-innovation-group

Genetic analysis of samples from miscarriages using a new efficient molecular testing strategy

Summary

Around 15% of pregnancies end in miscarriage; identifying the cause of pregnancy loss is important for couples and may be critical for the management of their future pregnancies.

The finding of chromosome imbalance in samples from miscarriages can provide a reason for pregnancy loss, may predict the risk of recurrence of miscarriage and inform reproductive options for couples.

Genetic investigation of recurrent miscarriage (defined as three or more miscarriages) is funded by the NHS.

Viapath's combined QF-PCR/array CGH approach detects more abnormalities than previous techniques.

Testing of samples from miscarriages?

Traditional testing of miscarriage products has involved culture of tissue, followed by G-banded chromosome analysis, a technique used to visualise the number and appearance of the cells' chromosomes. However, this approach is labour-intensive, has a high failure rate (because tissue samples often do not grow in culture) and can only detect large imbalances of >10Mb in the chromosomes. Viapath's Genetics Laboratory has replaced this methodology with a combined molecular approach using quantitative fluorescent polymerase chain reaction (QF-PCR) and array comparative genomic hybridisation (CGH) and have recently described the testing of more than 3,000 samples (Donaghue et al 2017).

Viapath's new testing strategy

The new strategy involves two steps:

Step 1. QF-PCR

QF-PCR is a cost-effective, fast, semi-quantitative technique in which microsatellite regions of chromosomes are amplified by PCR. This assay will detect the most common chromosome abnormalities in miscarriage samples which are aneuploidy (an abnormal number of chromosomes in a cell) for chromosomes 13, 14, 15, 16, 18, 21, 22, X and Y. Examples of the abnormalities detected are trisomy (three copies) of chromosome 21 (Down syndrome), three copies of all chromosomes (triploid) or one copy of the X chromosome (Turner syndrome). All abnormalities detected by this assay are likely to have caused the miscarriage (see figures 1 and 2 for examples of an abnormal result and abnormalities detected) .

This assay detects an abnormality in approximately 25% of samples.

Around 15% of pregnancies end in miscarriage, identifying the cause is important and may be critical for the management of future pregnancies

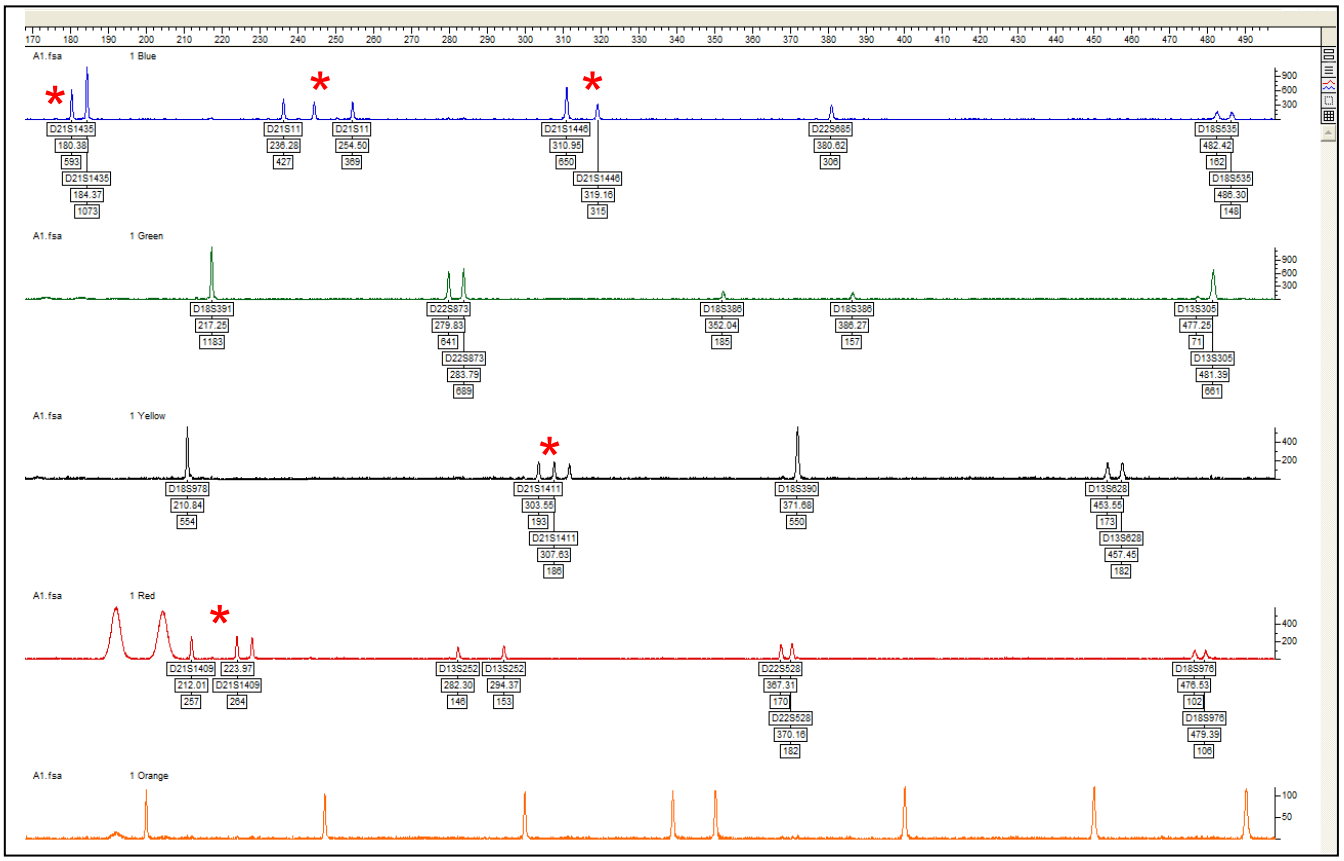
Step 2. Array CGH

If no abnormality is detected on QF-PCR then array CGH testing is also carried out. This test will pick up more abnormalities than QF-PCR but it is more labour-intensive. It scans for imbalances across the whole genome and detects imbalances >0.12Mb. All imbalances >1Mb are reported and any clinically significant imbalances which are <1Mb are reported. An abnormality is detected in approximately 10% of samples. Abnormalities include whole chromosome aneuploidies, unbalanced translocations, novel large deletions and duplications, small well known submicroscopic deletions and duplications and variants of unknown significance (VOUS).

Imbalances which are not reported

All imbalances >1Mb are reported, however imbalances <1Mb are only reported if the imbalance has a known fully penetrant clinical phenotype. Variants of unknown significance (VOUS) are not reported; the clinical significance of these imbalances and any potential relevance to the miscarriage is currently unknown.

Neurosusceptibility regions (which confer an increased risk of neurodisability) are also not reported; these imbalances will not have caused the miscarriage and their impact on the clinical features of any future children cannot be predicted.



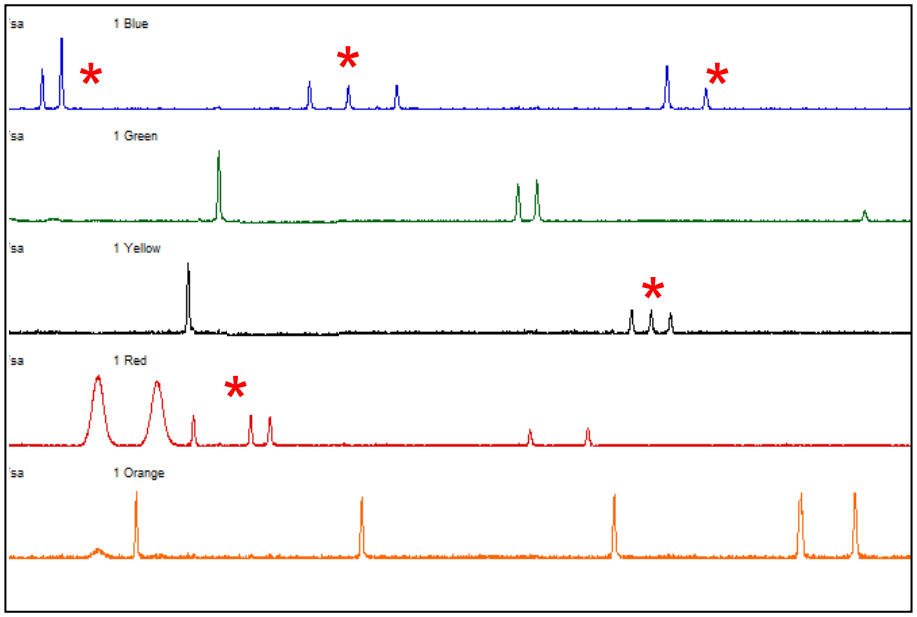


Figure 1: Example of an abnormal QF-PCR result: The asterisks show the microsatellite markers which are located on chromosome 21; this sample has trisomy (three copies) for chromosome 21.

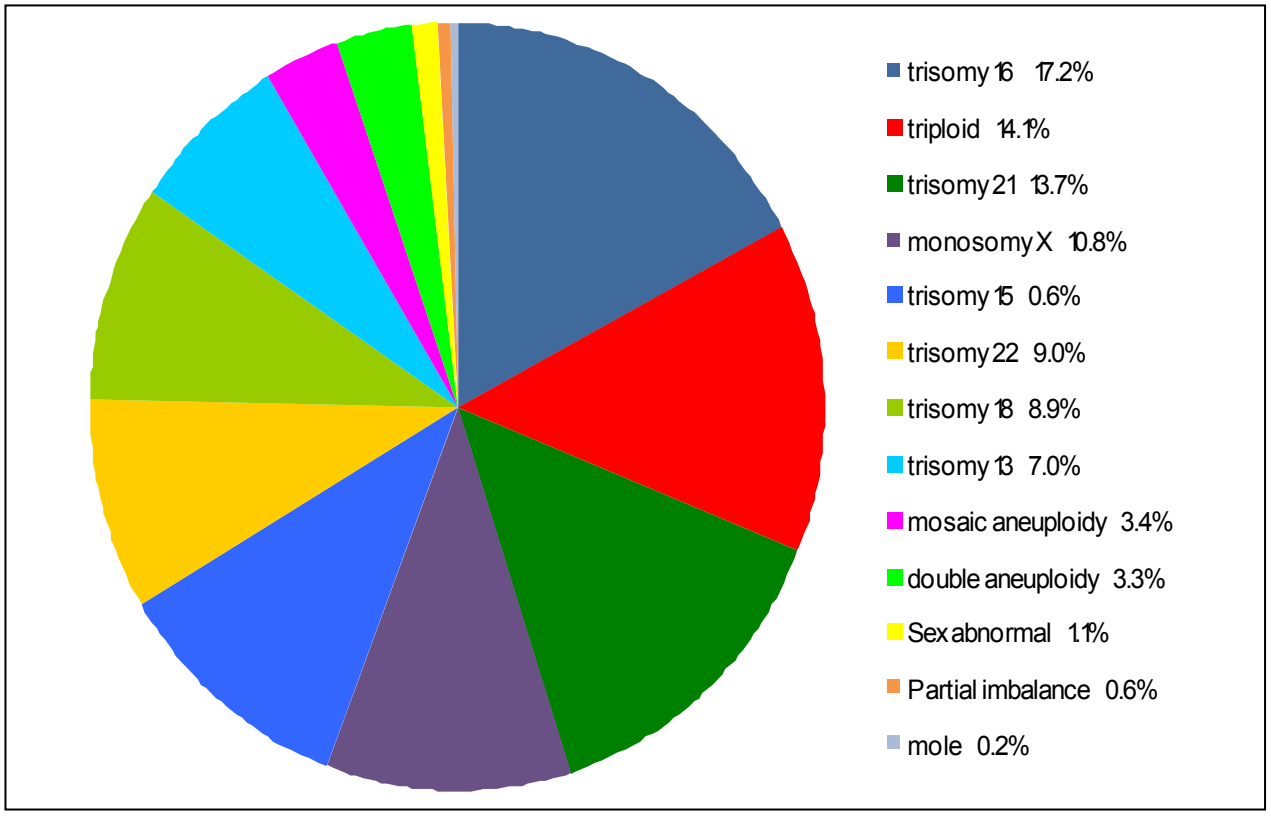


Figure 2: Total abnormalities detected by QF-PCR

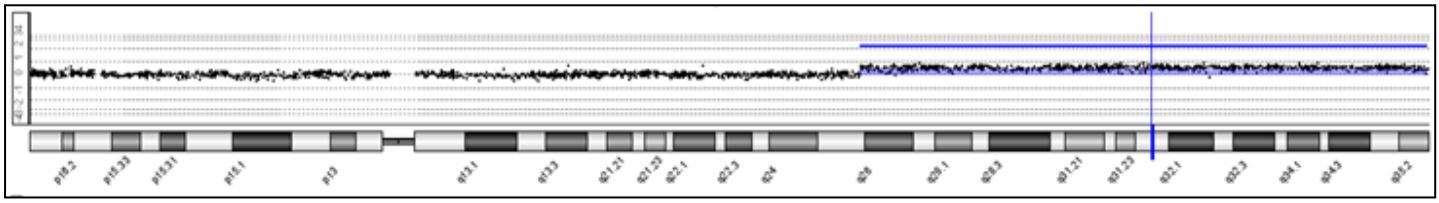


Figure 3: Example of an abnormal array CGH trace: Each dot represents a single probe on chromosome 4. In the region highlighted in blue all probes have a higher ratio indicating that this sample is duplicated for half of the long arm of chromosome 4

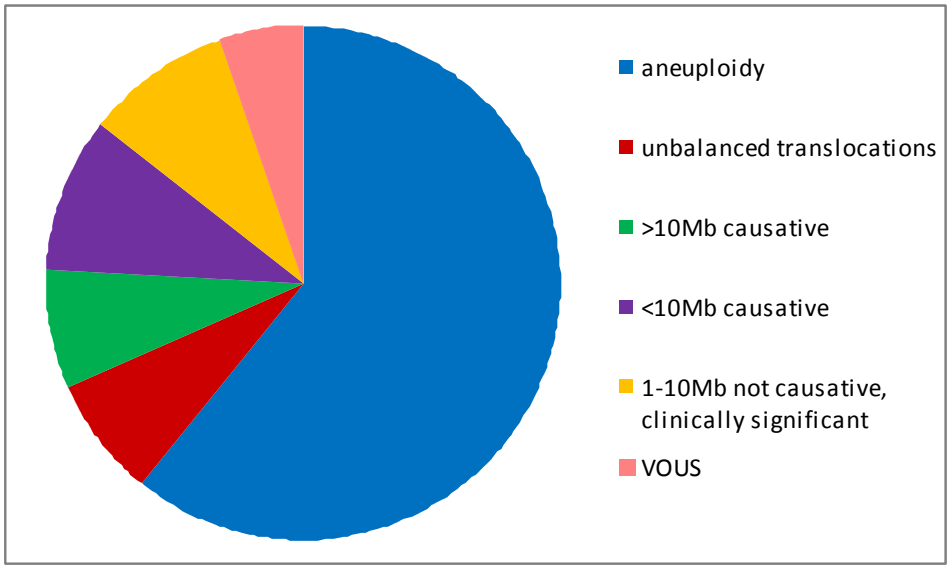


Figure 4: Total abnormalities detected by array CGH

Conclusion

This new strategy has a lower failure rate (1.4% compared to 30% for G-banded chromosome analysis) and reporting times are improved (89% of samples are reported within the target of 28 days compared to 79% for G-banded chromosome analysis). The new strategy also detects more imbalances, therefore the cause of the miscarriage is identified for more couples.

How can testing be obtained?

- Samples should be sent to Viapath's Genetics Laboratory at Guys Hospital
- Samples are accepted from any centre. Testing is funded by the NHS if a patient has had three or more miscarriages; otherwise privately funded samples are accepted.
- Samples should be sent in a dry sterile container and ideally should arrive within 24 hours. Samples do not need to be shipped on ice but should NOT be formalin fixed.

Testing is usually completed within 28 days

Reference:

Donaghue C et al 2217. Efficient and cost-effective genetic analysis of products of conception and fetal tissues using a QF-PCR/array CGH strategy; five years of data. *Mol Cytogenet.*