

Diagnostic microbiology: laboratory life but not as we know it

Valerie Bevan reports on behalf of the British Society for Microbial Technology from its recent annual scientific meeting, held at PHE Colindale in London in May.

Over 160 biomedical and medical scientists attended the annual British Society for Microbial Technology (BSMT) symposium in Colindale addressing changes in microbial technology and their implications for laboratories. Professor Eric Bolton chaired the meeting which covered various aspects of genomics, enteric PCR testing, MALDI-TOF MS, direct testing of specimens (metagenomics) and questioned whether total laboratory automation is a possibility. The presentations below are available on the BSMT website.¹



Eric Bolton

BACTERIAL GENOMICS AND METAGENOMICS

Professor Mark Pallen, Professor of Microbial Genomics and head of the microbiology and infection unit at Warwick Medical School, opened the day commenting that microbial genomics is in a state of permanent revolution. He asked how many of the complex processes in microbiology could be replaced by genomics in one form or another. Professor Pallen explained that molecular typing had been useful in identifying serial clonal outbreaks but was less useful in identifying transmission routes and mode of spread. By contrast, whole-genome sequencing provides unparalleled resolution between isolates, capable of pinpointing patient-to-patient transmission and

transmission from the environment. Professor Pallen explained how whole-genome sequencing is becoming increasingly accessible, particularly with the arrival of benchtop sequencers, and is now in routine practice in some parts of public health microbiology e.g. TB microbiology. He described an interesting case study in open-source genomics, which included crowd-sourced bioinformatics analyses. He reported that that genomics had been used to show that mixed infections of tuberculosis were common in the 18th century.



Mark Pallen

Professor Pallen stressed that through diagnostic metagenomics sequencing could go beyond between the genomics of cultured isolates while retaining the open-endedness of culture in finding unknown unknowns. Looking to the future, Mark highlighted the

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challenges faced before diagnostic metagenomics can be employed in routine practice. Finally, he hinted that nanopore sequencing employing a palmtop sequencer is likely to transform the research landscape yet again and usher in new opportunities in diagnostic microbiology.

WHOLE GENOME SEQUENCING IN *S. AUREUS*

Dr N Claire Gordon, Clinical Research Fellow at the Nuffield Department of Medicine in the University of Oxford, discussed the role of whole genome sequencing (WGS) in the detection of *Staphylococcus aureus* which remains a major healthcare associated pathogen and cause of outbreaks. Dr Gordon hypothesised that WGS can provide all the information needed to diagnose, treat and investigate illness due to *S. aureus*. To date, WGS has been used to study outbreaks and person to person spread; predict antimicrobial resistance and to look for virulence factors. Claire noted the limitations of standard typing methods in outbreaks indicating that it can lead to wrong conclusions being drawn. In contrast, WGS provides much higher resolution in determining whether isolates are related.



Claire Gordon

WGS can be used for predicting antibiotic resistance but is unlikely to replace phenotyping entirely as it is unable to detect unknown or emerging resistance determinants.

Current processing requires several different types of equipment and kits and the process still starts with the need to differentiate organisms by Gram stain. By contrast, WGS can use the same platform to

provide the same information, potentially for all organisms. Although costs of WGS are decreasing, standard investigation is still approximately half the price of WGS.

Looking to the future, like Professor Pallen, Dr Gordon indicated that palm top nanopore sequencing will become available for WGS, along with efficient, user-friendly software, making it much more accessible for routine laboratory use. Dr Gordon concluded that for *S. aureus*, WGS has greater resolution than current standard typing for outbreak investigation. WGS data reliably predicted antimicrobial resistance for majority of isolates tested, with overall error rates equivalent to current phenotyping tests, and cost and turnaround time for WGS are approaching those of standard typing.

MALDI-TOF MS IN CLINICAL MICROBIOLOGY

Dr Jette Jung, Consultant in Clinical Microbiology, University of Munich, discussed current and possible future applications of MALDI-TOF MS. MALDI-TOF is fast, providing a result within minutes compared to overnight with traditional microbiology; it is highly accurate and robust; there is an extensive database including fungi and mycobacteria and identification is possible direct from patient derived materials such as blood culture and urine. Dr Jung highlighted uses of this technology for other applications including further characterisation and sub-typing of bacterial isolates, resistance testing and epidemiology. She noted that MALDI-TOF has been used to distinguish between outbreak and non-outbreak strains (eg for *Legionella pneumophila*). Using the examples of highly virulent group A and B streptococci she illustrated the potential of MALDI-TOF technology to identify strains with certain biological characteristics. In terms of resistance testing several approaches were presented. For MRSA, MALDI-TOF MS is capable to discriminate between major multiresistant *S. aureus* (MRSA) lineages and initial publications suggest that in some cases detection of purified β -lactamases might be possible. However, the functional detection of β -lactamases is considered the most promising technique at the moment. Its use for rapid identification of Enterobacteriaceae resistant against third generation cephalosporins was presented as a particularly relevant application in the septic patient, where the assay can be performed directly from the blood culture flask.

Jette concluded that numerous publications show the potential of MALDI-TOF MS technology for characterization of bacteria below species level, however novel tools and criteria for data interpretation including more bioinformatics are needed.



Jette Jung

Prerequisites for the detection of β -lactamases in the routine laboratory (automated software/stable assay conditions) are available. In addition, other approaches for rapid resistance testing, e.g. metabolic activity assay and semi-quantitative assay (MS-ASTRA) are at an experimental stage.

APPLICATION OF PCR IN CLINICAL MICROBIOLOGY

Dr Kate Templeton, Consultant Clinical Scientist, Microbiology, Royal Infirmary of Edinburgh, opened her talk emphasising the importance of global diarrheal disease, second only to respiratory infections in the numbers of deaths caused annually (2.2 million compared with 4.2 million, respectively). This leads in Edinburgh to a workload of 30,000 enteric cultures every year. The microbiology service faces many challenges in meeting this demand including an ever increasing workload, fewer staff, skilled staff retiring and ever reducing budgets. This is countered by the continuing need to improve the clinical service, to ensure equity of access for patients with patient centred care, and the need for fast and accurate results.

Dr Templeton reported that PCR, which first became available in 2005, is now the gold standard for three important parasitic infections: *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium parvum*. She then showed that PCR is an option for other enteric infections such as norovirus and *Clostridium difficile* and showed that PCR could be used to undertake the same norovirus workload with the same budget but with fewer staff. She presented the validation results for norovirus using the BDMAX, an integrated extraction and PCR platform which was evaluated in 2012 and introduced into her laboratory in 2013. The outcomes for the service were that BDMAX requires less technical skill and hands-on time to perform the test and analyse results and is currently run by biomedical scientist support staff at bands 2 and 4 with seven-day testing being available. Turnaround times have improved to four hours, 17 minutes with 70–80% of results received by the infection control team by 15.00 hours. In addition, weekend testing contributes to advising on ward closures and there is a decrease in lost beds. Multiplex PCR testing for *C. difficile* is carried out on the same platform with the same staff at a similar consumable cost to the previous glutamate dehydrogenase method.

Dr Templeton went on to present validation results for xTAG Gastrointestinal Pathogen Panel, a multiplex assay for simultaneous detection of bacterial, viral and parasitic causes of infectious gastroenteritis, showing 10 additional positives detected for norovirus with 29 dual infections, five triple infections



Kate Templeton

and one sample with four pathogens.

Kate then presented comparisons of BDMAX (Enteric Bacterial Panel plus Enteric Parasite Panel), Luminex (xTAG GPP) and Serosep (EntericBio realtime Gastro Panel 2) against a panel of *Salmonella*, *Shigella*, VTEC, *Campylobacter*, *Giardia* and *Cryptosporidium*. Results on retrospective samples have been undertaken and the next phase is to test in parallel one system for two months with culture methods and to report in parallel with the LIMS system.

Finally, Kate gave some advice about undertaking evaluations: the main requirement is to address the clinical problem. All systems should perform satisfactorily in retrospective testing before doing prospective testing. Costs must be calculated but making the case within one's own resources is one of the main reasons for success. Finally, Kate advised: Be imaginative!

GENOMICS: IMPACT ON DIAGNOSTIC MICROBIOLOGY

Professor Rick Holliman, PHE Lead Public Health Microbiologist for London, gave a comprehensive overview of the impact of genomics in diagnostic microbiology. He discussed the current situation, anticipated the future, predicting how microbiology will change and how it will affect those of us who work in laboratories. Rick stated that current microbiology is reactive with extended turnaround times and most microbial tests have limited direct impact on patient care. The application of genomics will change this by improving identification of pathogens, predicting susceptibility and determining epidemiology; turn-round-times will be reduced, there will be increased profiles, decreased costs, enhanced safety and eventually near patient testing will be the norm.

Professor Holliman suggested that applying genomics in clinical practice means selecting the appropriate pathogens for testing, prioritising with a systematic deployment plan, determining genomic diversity, applying due diligence and undertaking initial parallel testing (both phenotypic and genotypic). Determining the genomic stability of a pathogen is carried out in a number of sequential steps: testing the technical reproducibility by resequencing a single sample; testing multiple samples from one individual at one time; multiple samples from one individual over a period of time; multiple samples from a point source outbreak; and finally multiple samples from the community.

He continued by saying that applying WGS in clinical practice means changes for laboratories in equipment, information technology and LIMS links, bioinformatics, staff training, assay validation (generic and specific), local



Rick Holliman

verification, UKAS accreditation (addition to scope), internal and external quality assurance programmes. To achieve all this involves a lead-in time with parallel testing (phenotypic and genotypic) of several months (possibly six to 12 months).

Rick emphasised the need to find out what is already available locally to enable cooperation with other clinical services where progress with genomics may already have been made. This could lead to sharing staff, standing operating procedures, validation and sharing other quality assurance data to help prepare for accreditation. Implementation of genomics into diagnostic laboratories means finding different ways of working with a different skill mix. Although staff may feel threatened by these changes, there are benefits for staff in obtaining new skills and competencies which will provide enhanced employment opportunities through transferable skills. The advantages for the patient are much reduced turn-round-times and enhanced clinical impact.

Professor Holliman highlighted where genomics needs developing. Reliable direct genomic investigation of clinical specimens (metagenomics) is much called for with real-time diagnosis. Such information will improve effective antimicrobial stewardship and guide immediate infection control interventions.

In conclusion, Rick reiterated that current microbiology methods are slow, restricted and have limited direct clinical impact and predicted that first-wave genomics will be deployed within six months. Finally he commented that genomics will have a profound impact on diagnostic, specialist and reference laboratories, but envisaged that microbiology will change rather than disappear.

TOTAL LABORATORY AUTOMATION: THE REAL POTENTIAL

Denise Cook, General Manager, Department of Infection and Immunity Sciences, Frimley Health NHS Foundation Trust, challenged the audience to think differently about automation and to use it to improve and develop the pathology service. She noted that automation has led to pockets of efficiency but its implementation is fragmented and 'total automation' is not available yet. She then commented on the Review of Pathology Services,² chaired by Lord Carter of Coles, observing his recommendation to consolidate laboratories to improve efficiency, safety and quality. Ms Cook noted that there have been considerable changes following the Carter report with changes in commissioning, increased input from the private sector and reductions in public funding to laboratories. In addition there is a shift from secondary to primary care and an increased quality agenda.

Denise presented several reasons for change including the changing model of care

'In order to develop a patient-centred service, laboratories need to understand demand and respond to it by examining and improving their service configuration'

across the whole health economy with fewer acute and more community based services; the need for greater efficiency in order to remain competitive; the advent of advanced automated technology giving efficiency and productivity gains; and point of care testing which will give greater flexibility and adaptability of service provision to smaller units. To undertake such changes and develop a patient centred service, laboratories need to understand demand and respond to it by examining and improving their service configuration. There will be a need to re-profile the workforce in line with the new technology in order to make more efficient use of capacity and to link this with appropriate information technology systems. Denise submitted that the use of instrument automation and robotics alone does little to improve pathology services if introduced in isolation and needs to be part of an overall strategy for pathology which includes pre and post analytical systems and which takes account of other clinical services.

To reap maximum benefit, Denise considered that there should be a national specification for pathology services but did not speculate who might take that role on.

DIRECT DETECTION OF BACTERIA FROM CLINICAL SPECIMENS

Dr Mark Wilks, Lead Clinical Scientist, Barts Health NHS Trust, London, described the Abbott Iridica system, the first system to allow the direct detection of most pathogens from a clinical specimen such as blood, CSF, BAL or joint fluid within six to eight hours without any culture. This therefore represents a decisive break from conventional microbiology which relies on culture or enrichment of some kind to obtain enough biomass to identify the organism of organisms. The technology has taken over 10 years and millions of dollars to develop to a state where it is robust enough to use in the diagnostic microbiology laboratory, although the basic principles are relatively simple.

The Abbot Iridica system consists of a panel of individual and multiplexed PCR reactions which are applied to each specimen after DNA extraction. However, instead of identifying the resultant products by sequencing in the normal way, the products in the PCR reaction mix are desalted, electrically charged and separated by weight in a mass spectrometer. In this case the mass spectrometer is an electrospray ionisation

mass spectrometer (ESI/MS) and not the MALDI-TOF type that microbiologists have become familiar with over the last few years. Hence, the general term for the approach – PCR ESI/MS. Identifying the components of the amplified DNA relies on the fact that each DNA base has a fractionally different weight. So a product that has been found to have a mass of 30,381.72 atomic mass units in the mass spectrometer, can only have a composition of 27 adenosine, 30 guanine, 21 cytosine and 21 thymine residues. The results are then compared automatically by searching a database of predicted PCR product masses for a wide range of bacteria, fungi and viruses for a match. This process happens automatically in a matter of minutes and a report generated with the name of an organism and its approximate level in the original specimen.

Mark described the results of a multi-centre trial using the Iridica for the rapid detection of sepsis in nine different adult ICUs across Europe. The results look highly encouraging with the technology capable of detecting a much wider range of pathogens than could be cultured as with many other nucleic acid detecting technologies. Barts Health NHS Trust is the first centre in the world to use the Iridica in routine diagnostic use. Although the technology has produced some amazing results such as the detection of *Mycobacterium tuberculosis* DNA direct from blood, it may be that its main use could be in ruling out infection quickly. Instead of searching for pathogens one by one, over a matter of days and sometimes weeks, it might allow infection to be ruled out altogether as a cause of the patient symptoms, allowing antibiotic treatment to be stopped or not started and other causes of illness examined. ■

REFERENCES

- 1 BSMT website (www.bsmt.org.uk).
- 2 Carter P. *Report of the Second Phase of the Review of NHS Pathology Services in England*. London: DH Publications, 2008.

The BSMT Autumn Symposium *To Screen or Not to Screen* will be held on Friday 27 November 2015 at the Liverpool Maritime Museum. Professor Eric Bolton and Professor Brian Duerden will chair the meeting. The Keynote speaker will be Dr Helen Lee, Director of Research, School of Clinical Medicine, University of Cambridge.



Denise Cook



Mark Wilks