Rapid diagnostics – time is relative: a BSMT Annual Scientific Meeting report

Mark Wilks reports on the Annual Scientific Conference of the British Society for Microbial Technology, held at Public Health England, Colindale, London, earlier this year.

Nearly 130 microbiologists, consisting mainly of biomedical scientists, clinical scientists and medical microbiologists, attended the Annual Scientific Conference of the British Society for Microbial Technology (BSMT) at PHE Colindale in May. The focus this year was on rapid diagnostics and how current technological developments are impacting on the medical microbiology laboratory, and will continue to do so.

Optimising recovery of bacteria from blood cultures

Professor Eric Bolton (President of the BSMT) chaired the morning session, and introduced the first speaker, Dr Mike Weinbren (Consultant Microbiologist, Kingsmill Hospital, Sherwood Forest NHS Foundation Trust). In some ways distinct from the other presentations, this talk focused on how to get the maximum value out of present methods, rather than introducing new molecular-based technology.

Before laboratories consider offering rapid matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) or expensive rapid panel-based molecular blood culture (BC) diagnostics, they should optimise pre-analytical, analytical and post-analytical processes and procedures surrounding BC systems. This sobering presentation showed how far we still fall short in the NHS in handling a specimen which everyone agrees is valuable. For example, although it's been known for several decades that the amount of blood collected is critical, this work showed that in only a quarter of cases is the volume of blood collected considered to be adequate. Again, two sets of blood cultures are recommended for adults but this is often not performed, reducing the overall yield significantly.

Having taken the blood culture, although everyone is aware of the need to ensure that it is placed in the analyser as soon as possible, in practice the time taken is incredibly long. Many hospitals leave blood cultures on the wards, or once they reach the laboratory they sit around at room temperature before being put in the analyser.

Dr Weinbren explained how, in his hospital, cooperation with the adjacent haematology laboratory which runs a 24-hour service was hugely important. Siting the blood culture analyser in haematology meant that when cultures were received they could be incubated immediately at any time of the day or night. This was accompanied by an extensive publicity campaign in the hospital on the importance of correctly taking blood cultures and the need for their rapid transport and frequent audit to make sure that the new procedures once put in place were being followed. The result was that substantial reductions in the time to positivity of blood cultures were achieved.

The reference to an excellent publication cited by Dr Weinbren on this topic, and freely available online, is given at the end of this report.

Device-related orthopaedic infections and the potential place of new technologies

Next, Dr Bridget Atkins (Consultant in Microbiology and Infectious Diseases and a physician in the Bone Infection Unit [BIU] in Oxford) gave a fascinating overview of the whole process of the

Following each session, a lively panel discussion ensued with good input from members of the audience.

diagnosis and treatment of bone and joint infections. This focused not just on the usefulness of newer techniques such as sonication and molecular methods, but also on the whole process of managing these complicated infections and the need for a strong multidisciplinary team.

Patients with complex bone and joint infections often have unsatisfactory interactions with the healthcare profession, sometimes having the wrong diagnosis, the wrong orthopaedic surgery, poor soft tissue management, and the wrong antimicrobial treatment. Even if three out of four elements on this list are correct, the outcome may still be poor. Sometimes all the elements are correct but they have not all been done at the same time. Patients may end up with multidrug-resistant organisms, drug side-effects, pain, disability, leaking wounds, long hospital stays, depression and disillusionment with the medical profession.

Getting the diagnosis right is important – both anatomically and microbiologically. Risk factors for less-common causes of osteomyelitis should be determined and an interactive relationship with the laboratory developed. Pre-operative biopsies can sometimes be considered; however, meticulous intraoperative sampling, good laboratory processing and accurate interpretation of results are crucial.

No laboratory test will work well if surgical sampling is not expertly performed. The patient should be off antibiotics and the procedure performed with no-touch technique using separate sterile instruments and from multiple sites. Histopathology should always be performed.

In the microbiology laboratory, prevention of contamination while adequately disrupting biofilm, using enriched media, culturing for an adequate duration and performing appropriate identification and antibiograms are vital to an accurate diagnosis. Soft tissue management is as important as the bones, so plastic surgeons need to be an integral part of the team. Intraoperative and post-operative antibiotic therapy should be rational and managed with skill – while not causing harm to the patient.

Clinical progress must be adequately monitored. Failure means a complete re-assessment, not simply prolonging antibiotics. Much of this should be delivered through multidisciplinary specialist bone infection units where infection doctors manage the triage of referral, in-patient care, antimicrobials and discharge planning, working closely with their surgical colleagues.

Although data from a number of studies using sonication and molecular tests were presented, Dr Atkins thought that at the moment it is still too early to advocate the introduction into routine use, as there was no obvious single measure or set of measures which gave greatly improved results.

Tracking *Staphylococcus aureus* around the intensive care unit

Staphylococcus aureus infections in ICU have long been a problem. With this in mind, after the morning break Dr Stephanie Dancer (Consultant Microbiologist, NHS Lanarkshire, and Professor of Microbiology, Edinburgh Napier University) summarised studies over several years on the dynamics of the spread of *S. aureus* between surfaces, such as furniture and equipment, patients, staff hands and air in ICU.

A wide variety of different techniques was used including air sampling using slit samplers and settle plates, double-sided dip slides which could be applied to different surfaces in the laboratory, swabbing the hands of staff and covert observation to see how often surfaces were touched by staff. Not surprisingly, frequently touched surfaces demonstrated higher amounts of microbial contamination.

Whole-genome sequencing of the isolates was used to show transmission around the unit. Interestingly, in 34 transmission events involving identical strains, 22 (65%) were autologous; so nearly two-thirds of ICU-acquired *S. aureus* infection originated from the patient's own flora. In one sense this is encouraging as cross-transmission between patients, at least in this ICU, was quite rare (9% of episodes); however, the study did show extensive contamination sites which were frequently touched such as bed rails and intravenous (iv) pumps, and clearly more attention needs to be paid to preventing this contamination from occurring; after all, if you are a patient on ICU and you acquire an *S. aureus* infection it is not really relevant whether it's your *S. aureus* or one from another patient.

Dr Mike Weinbren discusses aspects of his blood culture work with Michael Croughan, BSMT Hon Treasurer, and a BSMT delegate.

BSMT President Professor Eric Bolton in conversation with Dr Robert George, founder member and inaugural BSMT President, and previously Head of the Respiratory and Systemic Infection Laboratory at Colindale.

It's hard to say how generalisable these results are, but there seems no reason to suppose that the ICU studied was different from many others, and Dr Dancer described strikingly similar results from a study of vancomycin-resistant *Enterococcus* in an ICU in a tertiary Australian hospital where whole-genome sequencing was used. Here, patients contaminated environmental sites and environmental sources, which not only led to patient colonisation but also to infection.

Respiratory virus detection: increased efficiency to meet winter demands

To conclude the morning session, Dr Gemma Clark (Clinical Scientist, Nottingham University Hospitals) described the implementation of a commercial multiplex polymerase chain reaction (PCR) method for the detection of respiratory viruses. With their seasonal variation and unpredictable demand for testing from year to year, this presents a particular challenge for the diagnostic laboratory.

Several years ago the laboratory faced a number of major challenges, including the loss of experienced staff, an increase in workload, and technical problems with the in-house assays that were in use. These problems included master mix quality control failures and failures of positive controls. There were also concerns over the performance of the actual assays. There were more external quality assessment (EQA) failures than deemed acceptable, and worries over primer sensitivity and specificity. Workflow analysis showed the whole process was quite 'messy' and it was clear that the way the whole process was being carried out was not efficient.

Dr Clark described the implementation of a commercial assay produced by Aus Diagnostics which allows the whole

workflow to be simplified, reduced in complexity and the quality greatly improved. This particular test adopted produced semiquantitative results (copies/10 µL) which are not strictly comparable to the Ct values obtained by real-time PCR; however, they were useful to monitor trends and fall in values as patients responded to antiviral therapy. Despite a 28% increase in workload annually, turnaround times were maintained, there were improvements in EQA performance and improved staff satisfaction. Further improvements which are intended to be rolled out in the winter of 2018 included the possibility of point-of-care testing in the A&E department to reduce turnaround times still further.

Molecular diagnostics for enteric infections – relevance for clinical diagnosis and public health

Professor Brian Duerden CBE chaired the afternoon session and introduced the first speaker, BSMT President Professor Eric Bolton (Honorary Professor in the Department of Epidemiology and Population Health, University of Liverpool), who described initial findings from what is the largest study so far carried out on the use of molecular diagnostics for enteric infections and looked at the relevance for clinical diagnosis and public health.

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Gastrointestinal disease is still a major problem in the UK and studies over the last 10 years have shown that there are approximately 17 million cases of infectious diarrhoeal disease in the UK each year. This burden of infection is also associated with a significant economic cost to those affected and the UK economy of greater than £2 billion.

Current surveillance systems are based on both syndromic and laboratory-confirmed data but there are weaknesses with both systems. Furthermore, there is no proactive system for the rapid recognition of outbreaks of disease in the community. Rapid laboratory confirmation is limited by the use of a multiplicity of traditional methods usually targeted at specific pathogens and applied in many laboratories with restrictive algorithms. These can be both slow and give misleading results. There are also several enteric pathogens that cannot be detected by these traditional methods.

In an attempt to address the limitations of the current surveillance and diagnostic methods a novel research project was developed. This is known as the 'Integrate Project – Fully integrated, real-time detection, diagnosis and control of community diarrhoeal disease clusters and outbreaks'. The main objectives of this study were to:

- create a new, one-health paradigm for detecting and investigating clusters and outbreaks of diarrhoea and vomiting in the community
- develop a new approach to population sampling based on syndromic surveillance in the community and which involved a rapid system for cluster detection known as: Ascertainment and Enhancement of Gastrointestinal Surveillance and Statistics (AEGISS)
- evaluate the use of modern microbiological methods, including: a) rapid molecular diagnostics and b) the potential role of microbial genomics

- develop and integrate veterinary surveillance systems to monitor in real time links with human infections.

This project, led by Professor Sarah O'Brien from the University of Liverpool and undertaken in the North West of England, involved collaborators from the following organisations: Public Health England, Bangor University, Lancaster University, University of Surrey, Royal College of General Practitioners, Animal Health and Veterinary Laboratories Agency, Central Manchester University Hospitals NHS Foundation Trust, Lancashire Teaching Hospitals NHS Foundation Trust, the Royal Liverpool and Broadgreen Hospitals NHS Trust, and Luminex .

The study was divided into five work-packages and Professor Bolton's presentation concentrated on some of the findings from Work-Package 2 – 'Modern Microbiology' relating to laboratory diagnosis.

A total of 1945 faecal samples, submitted by collaborating general practices in the North West of England, were transported by post and tested in one of the three sentinel diagnostic laboratories. Each of the three laboratories carried out their routine investigation procedures and accredited methods for the laboratory diagnosis of community-acquired gastrointestinal infections. These methods included: bacterial culture, microscopy, immunoassays and in house and/or commercial molecular techniques.

In addition, microbiologists from each laboratory were trained to perform the Luminex xTAG Gastrointestinal Pathogen Panel (GPP) assays according to the manufacturer's instructions. The Luminex xTAG GPP involves a 21-multiplex molecular assay that simultaneously detects 15 enteric pathogens. The pathogens detected are shown in Table 1, which is reproduced by permission of Luminex. Previous studies in the UK have shown that sapovirus and enteroaggregative *Escherichia coli* are common community-acquired infections. Therefore, assays were developed and validated for the Luminex platform. Hence, in this study faecal samples were screened for 17 enteric pathogens by the Luminex assays.

Results of the assays were interpreted according to the manufacturer's instructions. One of the main objectives of this part of the Integrate Project was to compare the outputs from the routine diagnostic methods with those of the molecular system and to assess performance against a set of outcome-based indicators including: diagnostic yield, numbers of false-positive and false-negative stool samples, and time to detection of the results. The following is an overview of the performance characteristic of the routine methods and the Luminex xTAG assays.

Table 1. Luminex xTAG GPP targets.

Reportable targets	Number of analytes
Adenovirus 40/41	1
Rotavirus A	1
Norovirus GI/GII	2
Clostridium difficile toxin A/B	2
Salmonella	2
Shigella	1
Campylobacter (*C. Jejuni, C. coli, C. lari*)	1
Escherichia coli O157	1
Enterotoxigenic *E. coli* (ETEC) LT/ST	2
Yersinia enterocolitica	1
Vibrio cholerae	1
Shiga-like toxin-producing *E. coli* (STEC) stx 1/stx 2	2
Giardia lamblia	1
Cryptosporidium	1
Entamoeba histolytica	1
Internal control {MS2)	1
Total	**21**

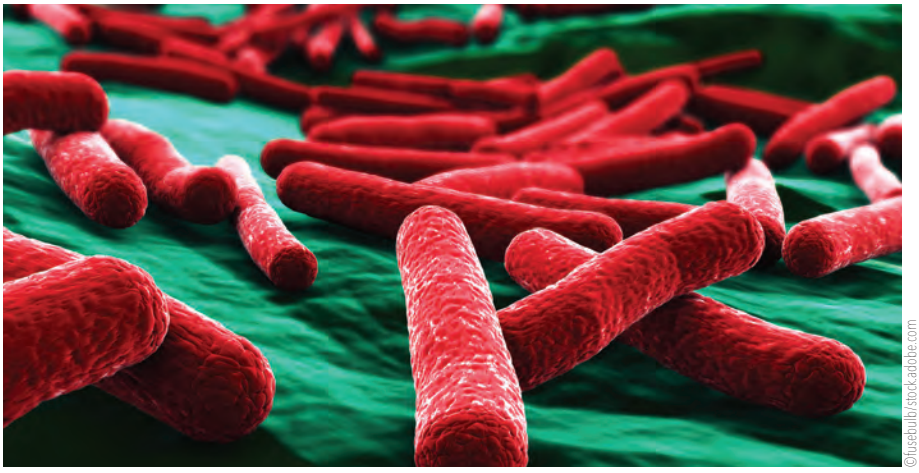
The diagnostic yields for the Luminex xTAG assays (including the assays for sapovirus and enteroaggregative *E. coli*) and the routine procedures were 45.5% and 13.4%, respectively. This is consistent with other published studies that have compared molecular methods to routine procedures.

The Luminex xTAG assays gave an overall positive predictive value of 83.3–100%. This range is given because it is not possible to determine the number of false positives because molecular methods are known to be more sensitive than traditional methods. The Luminex xTAG assays gave an overall negative predictive value of 96.1%.

Of the faecal samples tested and reported within one day by the Luminex xTAG assays the routine methods produced a test report within an average of 3.18 days.

For those microbiologists and laboratories considering implementing molecular screening for diagnosing gastrointestinal infections, Professor Bolton's following comments may be useful. There are some recognised potential issues with molecular (PCR) diagnostics:

- PCR methods may be subject to sample inhibition and hence no result can be obtained. This occurred with about 2% of samples in the above study.
- PCR methods may yield clinically false-positive results due to either increased sensitivity and/or lack of specificity. This is particularly associated with the detection of *Shigella*. species.
- PCR methods may produce false-negative results due to limitations



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Enteroaggregative *Escherichia coli* are common community-acquired infections, and assays have been developed and validated for the Luminex platform

of the gene targets. This can be a problem with the detection of all *Salmonella*-positive samples.
- PCR methods detect multiple pathogens in samples which may lead to difficulty in interpretation.

However, there are potential benefits of using molecular (PCR) diagnostics:
- PCR methods give similar or improved detection rates for many pathogens using a common approach.
- PCR methods detect organisms that are difficult or not possible to detect by routine/traditional techniques (eg enterovirulent *E. coli* and viruses).
- Results are more rapid than routine/traditional methods.
- These methods give some indication of potential pathogenicity because the PCR targets may be associated with toxin genes.

There is also a potential benefit for public health because using a multiplex approach to detect a wider range of pathogens may offer significant improvement for epidemiological surveillance.

Rapid metagenomics diagnosis of hospital-acquired infection

At last year's annual scientific conference Dr Kate Templeton from Edinburgh described her use of a range of multiplex PCR to find causative agents from a very high proportion of cases of community-acquired pneumonia. At this year's conference, a sequence-based approach was described. Dr Justin O'Grady (Senior Lecturer in Medical Microbiology, Norwich Medical School, University of East Anglia) focused on the rapid genomic diagnosis of hospital-acquired pneumonia using Oxford nanopore sequencing.

This fascinating technology uses a very small DNA sequencer about the size of a box of matches, which is plugged into a laptop or desktop computer. Long strands of DNA are separated by passage through pores in a membrane. As the strands of DNA pass through the membrane, different bases in the DNA develop different electrical charges and the sequence can thus be determined. The amount of preparation can be very small and only take a few minutes, and the first sequencing results appear in real time, allowing the world's first comprehensive trial of metagenomics in the diagnosis of infection.

The turnaround from sample to pathogen genome and antibiotic resistance results was approximately eight hours. One of the main problems to be overcome is that of sequencing pathogen DNA in a vast excess of human DNA; for example, in sepsis the approximate ratio of human to bacterial DNA is 10^9.

> A novel DNA-based microarray platform for the rapid detection of sepsis performed well, but the take-up of the test in diagnostic clinical microbiology laboratories was zero

There are two obvious solutions to this – either deplete the host DNA or enrich the pathogen DNA.

Dr O'Grady described his approach which concentrates on depleting the host DNA using a range of commercial and in-house strategies including differential centrifugation and differential lysis. This results in up to 99.9999% (10^6) removal of human DNA, allowing pathogen DNA to be sequenced as well as the inevitable remaining human DNA that is still present in excess.

The approach seemed highly encouraging – sequencing results were about 90% concordant with the detection of pathogens by culture. As with many molecular studies, more positive results were obtained than with culture. Antibiotic resistance genes were also detected and these correlated with those obtained by conventional methods.

Rapid diagnostics, bad bugs and antibiotics: not that easy

The final talk of the afternoon was given by Dr Vanya Gant (Clinical Director for Infection, University College London Hospital NHS Foundation Trust). This was a highly provocative review which looked back at some techniques that had not succeeded, and gave a tantalising glimpse of what the future might offer.

Dr Gant focused first on the importance of sepsis, which is of course well known, with 20 million cases per year worldwide, probably 135,000 deaths per year in Europe and 21,000 in the USA. There is clearly a desperate need for rapid, robust and reliable tests. He then described his evaluation of a novel DNA-based microarray platform for the rapid detection of sepsis. Although the system performed well, the take-up of the test in diagnostic clinical microbiology laboratories was zero, partly because of cost and partly because clinicians generally preferred to wait for the culture result.

Next he moved on to the problem of levels of bacteria detected and how to convert this into a useful report for a clinician. This is a particular problem with respiratory specimens where many of the pathogens are also part of the normal flora, and what a particular level of detection actually means may not be clear.

Unusually, Dr Gant highlighted the importance of the amount of space taken up by the introduction of a new piece equipment in the modern laboratory, where every square foot has to be accounted for. He pointed out that a large number of relatively small platforms have been developed in recent years to cope with this pressure, and although the performance is generally satisfactory it is not clear what difference they make to patient outcomes in the majority of cases.

Another problem with molecular platforms focuses on the detection of antibiotic resistance, and how the genotype can be related to the phenotype of the organism as measured in the laboratory. At present, there does not seem to be an answer to this problem.

Dr Gant's talk really summed up the whole day. While PCR-based or sequence-based technologies have the potential to increase the speed of diagnosis of bacterial infections considerably while reducing hands-on time, and the potential to reduce inappropriate antimicrobial prescribing and hopefully improve clinical outcome, but the performance in the clinic has yet to be established. Crucially, will clinicians trust the results?

Lively discussion

Following each session, a lively panel discussion ensued with good input from members of the audience. This interaction was carried through to the particularly strong and supportive trade show, which ensured an informative and varied symposium for delegates.

Reference

1 Banerjee R, Özenci V, Patel R. Individualized approaches are needed for optimized blood cultures. *Clin Infect Dis* 2016; **63** (10): 1332–9.

Dr Mark Wilks is a Clinical Scientist at Barts Health Trust, and Honorary Senior Lecturer, Barts and the London School of Medicine and Dentistry, Queen Mary University of London. Many of the presentations given may be accessed on the BSMT website (www.bsmt.org.uk).

The autumn symposium of the BSMT, entitled *Going Overboard with Microbiology – Women and Children First*, will be held at the Merseyside Maritime Museum in Liverpool on 19 October 2018. Details of the programme and an application form may be found online (www.bsmt.org.uk).