Rapid diagnostics – warts and all: a microbiology conference preview

This year's Annual Microbiology Conference of the British Society for Microbial Technology will be held at the RAF Museum in Hendon, North London, on 2 May. As usual, it will cover a range of different topics but this year there is a particular focus on the use of rapid testing in the diagnosis of infectious disease and the detection of antibiotic resistance.

To begin, it's worth restating the way in which the use of rapid diagnostic tests should help in the management of infection. In theory, rapid diagnostic tests (RDTs) can play a crucial role in antimicrobial stewardship by providing timely and accurate information about infectious diseases. Antimicrobial stewardship programmes (ASPs) aim to optimise the use of antimicrobial agents to improve patient outcomes, reduce resistance, and minimise adverse effects. Thus, RDTs enable rapid identification of the causative agents of infections, allowing healthcare providers to initiate targeted therapy to reduce the risk of inappropriate treatment. With precise information on the type of pathogen and its susceptibility, treatment plans can then be tailored to match the specific characteristics of the infection.

This personalisation should help avoid the use of overly broad-spectrum antibiotics, minimising the risk of resistance. Rapid diagnostic tests allow for a more targeted approach to treatment, reducing the reliance on empirical therapy. Reduced empirical antibiotic use in turn helps prevent the development of antibiotic resistance and minimises the impact on the patient's microbiome. Unnecessary use of antibiotics contributes to the development of antibiotic resistance so the increased use of RDTs should help identify cases where antibiotics are not required, preventing unnecessary exposure. This is particularly important in conditions where bacterial and viral infections may present with similar symptoms.

The conference provides scientists the opportunity to discuss and debate their experiences, and there will be ample opportunity to question the experts!

Rapid diagnostic tests provide information on antimicrobial susceptibility, allowing for the selection of the most effective antibiotic. Healthcare providers can avoid using broader-spectrum antibiotics when narrower-spectrum options are equally effective, thereby preserving the efficacy of antibiotics such as third-generation cephalosporins.

Quicker and more accurate diagnosis through the use of RDTs should in theory lead to shorter hospital stays by enabling prompt and effective treatment. Reduced hospital stays contribute to lower healthcare costs and decrease the risk of hospital-acquired infections. By facilitating appropriate and timely treatment, RDTs contribute to improved patient outcomes. Avoiding unnecessary antibiotic exposure reduces the risk of adverse effects and complications associated with prolonged antibiotic use, and RDTs should therefore be integrated into antimicrobial stewardship protocols to ensure their effective utilisation. Regular review and adaptation of stewardship guidelines

based on RDT results enhance the overall efficacy of the antimicrobial stewardship programme.

Defining rapid diagnostic tests

When summarised in a general way as above, it can be seen that the use of RDTs in the diagnosis of infectious disease must be a 'good thing'; however, the picture becomes a lot less clear when examined in any detail to try and look for high-quality evidence in support of a particular test or groups of tests and how they should be used.

For example, what is a rapid test anyway? There is in fact no agreed definition of what constitutes a rapid test. A rapid test is often understood as being any test that produces an actionable result in less time than that taken using a culture-based test. Others, however, tend to restrict use of the term to tests that are POC-based so the time taken to transport specimens to the laboratory and report results is removed from the equation, while yet others would consider the use of MALDI-TOF identification of bacteria that have been cultured, as a rapid test as it may reduce the time taken for identification and possibly AST determination by 24 hours.

Take the case of neonatal sepsis. Here, historically, the treatment approach has included early aggressive initiation of antibiotics because of the neonate's relative immunosuppression. Because early signs of sepsis in the newborn are non-specific, diagnostic studies are often ordered and treatment initiated in neonates before the presence of sepsis has been proven or excluded. Many asymptomatic neonates now undergo evaluation and are exposed to antibiotics. This approach has been questioned in recent years as more evidence emerges of the deleterious impact of unnecessary antibiotic exposure, including interference with the establishment of breast feeding, alterations in gut microbiome, increased incidence of childhood obesity and development of antimicrobial resistance, among others.

Astonishingly, among very low birthweight infants who were initially treated with antibiotics but subsequently found to have negative cultures, there is actually an increased risk of mortality and stage 3 retinopathy of prematurity. In other words, in addition to the well-described general risks of overprescribing as mentioned above, there is the possibility of causing direct and immediate harm to the infant who did not have sepsis to begin with.

Role of inflammatory biomarkers

There is often very little agreement on the performance even of tests like C-reactive protein (CRP) and procalcitonin (PCT), which have been in use for decades and are relatively cheap and easy to perform. At the BSMT meeting in May 2020, Professor Paul Dark, National Deputy Medical Director, NIHR Clinical Research Network, described the protocol for a multicentre randomised, controlled trial looking at the use of these two inflammatory biomarkers (CRP and PCT) in the management of hospitalised patients with suspected sepsis. While many ICUs use one or other of the tests, implementation has been highly variable and the evidence base underlying the implementation is often minimal or non-existent, just the belief that one or other of the tests is a 'good test'.

Many clinicians will vehemently support the use of one of these two tests while decrying the other based on their own experience or small trials. Indeed, it has been said that the only thing missing from the CRP test is the letter A. Professor Dark's trial (biomArker-guided Duration of Antibiotic treatment in hospitalised Patients with suspecTed Sepsis [ADAPT-Sepsis]) aims to finally provide the high-quality evidence on the usefulness of these two tests. The trial that Professor Dark described commenced in May 2017 and is due to finish recruitment in July 2024 – like many other trials, it has been held up by COVID-19. A total sample size of 2760 will be required to reliably detect a mean of one-day reduction in antibiotic duration. The trial will take up to six years, including follow-up, to produce a result.

Assessing the value of RDTs

If it has taken decades to get to the stage where we will definitively be able to determine the usefulness of CRP and PCT tests, how then should we evaluate the plethora of new tests being introduced, some of which will be on display in the exhibition at the BSMT meeting in May? To throw some light on this confusing picture, we are therefore particularly lucky to welcome Dr Luke Moore, Consultant Infectious Diseases, Microbiology, & Virology, Chelsea & Westminster NHS Foundation Trust, to give the introductory keynote speech on how we assess and value rapid diagnostics.

Dr Moore has been instrumental in setting up an expert working group from low-middle and high income countries to assess the value of RDTs in antimicrobial stewardship programmes and how they can be implemented in countries with vastly different resources. They have proposed the following global definition of RDTs for use in antimicrobial stewardship programmes: infectious disease RDTs include both microbial and host assays which can be conducted and actioned within a 24-hour period that can substantially support ASPs.

Sequencing revolution

Two years ago at this meeting, Adela Alcolea-Medina, Clinical Scientist from Guy's & St Thomas' Hospital, London, described her work on the development of rapid sequencing methods for the detection of respiratory pathogens, aimed at producing actionable results within eight hours rather than 24 hours. In those two years the methods have been refined and are now capable of

detecting all bacteria, DNA and RNA viruses and fungi in one simple, unified workflow. Additionally, bioinformatic analysis of sequencing data derived from metagenomics can predict antimicrobial resistance profiles, facilitating tailored treatment strategies and contributing to global efforts to combat antimicrobial resistance within the One Health framework. Results are obtainable within seven to eight hours. A trial of the use of the method is currently under way in several different ICUs in London, and she will present data on how the use of the method has helped patient management.

In theory, clinical metagenomics (CMg) has the potential to revolutionise infectious disease diagnostics by rapidly identifying and characterising all pathogenic microorganisms in clinical samples within a few hours. This should be particularly helpful in lower respiratory tract infections (LRTI) that are serious and can be caused by a broad range of known and emerging bacterial, fungal and viral pathogens. The current diagnostic approach using a combination of culture for bacteria and targeted PCR for viruses, supplemented by antigen detection and other tests, is often unsatisfactory. It requires multiple assays, provides staggered results, and ultimately incomplete information, which causes residual uncertainty around diagnosis and patient and antibiotic management. Moreover, samples are typically processed in separate clinical microbiology and virology laboratories and may need additional testing by reference laboratories if a rarer or fastidious pathogen is suspected. One stage that is crucial is depletion of the human DNA, which may be present in very high concentration. There are many challenges to achieving this objective, given the heterogeneity of human samples and the need to focus on identifying DNA and RNA pathogens that have many different sizes and structures and are nearly always present in low abundance compared with the host's cells.

Several human DNA depletion methods have been developed to increase sensitivity and reduce turnaround time (TAT) for detecting either DNA or RNA organisms in clinical samples using metagenomic techniques. These methods include physical separation by differential centrifugation, lysis of human cells using saponin, or other chaotropic agents. These methods allow bacteria, fungi or viruses to be successfully sequenced following human DNA depletion, but never all at the same time due to sample heterogeneity and fundamental differences in microbial cell morphology and abundance observed in clinical samples.

Session chair Professor Brian Duerden (left) in discussion with this year's speaker Ivor Mitchelmore in the trade show during a break in last year's conference programme.

In this novel approach, a unified metagenomic method utilises a mechanical human DNA depletion method that allows detection of bacteria, fungi and viruses present in a sample. It uses bead-beating to selectively disrupt human cells and release human nucleic acid for enzymatic digestion, prior to microbial cell lysis and nucleic acid extraction, followed by reverse transcription and DNA PCR amplification before real-time nanopore sequencing. The innovative approach involves a slow centrifugation step and mechanical lysis via bead-beating. This technique effectively depletes human DNA while preserving microbial DNA and RNA. Coupled with nanopore sequencing, this approach streamlines the workflow, providing clinically actionable information within a seven-hour timeframe.

This innovative metagenomic approach holds promise in overcoming existing limitations. It offers a technically straightforward method for robust respiratory pathogen detection. The prospect of a single, unified metagenomic test holds significant utility in clinical settings. It provides rapid and comprehensive results, informing initial treatment decisions, local infection control, and national surveillance. While challenges such as cost, validation and accreditation persist, this workflow represents a substantial step forward in unlocking the full clinical potential of metagenomics in infectious disease diagnostics.

This rapidly emerging field of RDTs using metagenomics in clinical microbiology presents both enormous opportunities but major challenges in implementation in the clinical laboratory. Addressing issues related to reagent DNA contamination, the removal of human DNA from specimens and cost barriers is imperative for the successful integration of metagenomics into routine clinical diagnostics. While continued research and technical improvements will take place, the major challenges are arguably now in the area of validation and standardisation to further establish the clinical utility of metagenomics, and ensure that it contributes to improved patient outcomes and public health to the fullest possible extent.

Nevertheless, we are conscious that for much of the world, there is no access to any laboratory facility for diagnostic tests, let alone RDTs. In complete contrast to the talk by Adela Alcolea-Medina, Ivor Mitchelmore, formerly chief biomedical scientist in microbiology at Luton and Dunstable Hospital, will talk about his experiences in helping to set up a laboratory from scratch in India, and how access to even the most basic blood culture system can have a major impact.

■ *This year's conference is dedicated to the memory of Mahsa Amini, an Iranian student about to start a degree in microbiology but was arrested on 13 September 2022 for 'improperly wearing the mandatory head covering'. She died in police custody several days later.*

There will be another article written by BSMT chairman Dr Mark Wilks in the April issue of *Pathology in Practice* looking at some of the themes of the other talks. As always, this year's BSMT conference will have a full trade show with 20 of the most innovative companies attending to present the latest developments and newest equipment to talk to delegates about what their company can offer laboratories.

The conference provides scientists the opportunity to discuss and debate their experiences and there will be ample opportunity to question the experts! Registration is now open. Book now to catch the early bird rate! Check the BSMT website (https://www.bsmt.org.uk) to register and for updates to the programme.

www.bsmt.org.uk