# The Genomic and Microbiology Revolution: In Technology We Trust?

Last year's annual conference of the BSMT took place in May as a webinar, inevitably on COVID-19. This year the BSMT decided to postpone the conference until July 2022 to maximise chances of holding a face-to-face event. Here, Mark Wilks, on behalf of the BSMT, provides a preview.

'In technology we trust?' is the subtitle of our conference and the justification for this slightly sceptical tone is nowhere clearer than in the field of sepsis diagnosis, the topic covered by our first speaker Professor Paul Dark from Manchester, where he is Professor of Intensive Care Medicine and also Deputy Medical Director of the National Institute of Health Research Clinical Research Network (NIHR CRN). Professor Dark spoke several years ago at our conference in 2017 and we are very pleased to be able to welcome him back to review progress made since then.

It is sobering to reflect that despite advances in health technology the mortality rate associated with sepsis is still around a third. Early recognition of sepsis is still a formidable challenge as the clinical signs and symptoms seen in a variety of acute conditions are not necessarily anything to do with infection. There is no agreement even over the value of tests such as C-reactive protein (CRP) and procalcitonin (PCT), which have been around for a very long time and the subject of numerous trials.

Indeed, in a previous talk to the BSMT, another intensivist suggested that the only thing missing from the CRP test was the letter A! In contrast, other centres value this test highly and consider monitoring CRP levels in combination with the results of other tests and their own clinical judgement to be really important in monitoring patient progress.

The current clinical paradigm in suspecting sepsis is to recognise that a patient is unwell with new organ dysfunction, and to consider if an infection could be the cause. Professor Dark has been involved in a large study evaluating the value of PCT and other emerging molecular diagnostic technologies, some involving nanoparticles and point-of-care tests in the setting of sterile tissue injury and severe infection.

It may be that the rapid development of sequencing directly from blood, which is discussed below, may provide a paradigm shift in this field as it will provide an unambiguous answer to whether or not there is an infection, the name of the organism and possibly some information on its resistance markers.

## Sequencing

One field in which sequencing has already shown its value is in the study of the COVID-19 pandemic, not just in the rapid detection of new variants but in understanding COVID-19 outbreaks in long-term care facilities, a subject studied by Dr Dinesh Aggarwal, Wellcome Clinical PhD Student, Department of Medicine, University of Cambridge. Here, high-resolution and accurate sequencing is essential and has shown that staff and residents in long-term care facilities tend to be infected with identical or very similar SARS-CoV-2 genomes. Combining this knowledge with local epidemiology studies allowed probable transmission routes to be better characterised.

Much of this work was based on Illumina sequencing; however, there has been increasing use of Oxford Nanopore technology (ONT). This small-scale sequencing platform, which is compact and has no capital cost, has enabled many laboratories especially in developing countries to perform their own rapid sequencing without being dependent on the need to send samples to central laboratories, or indeed overseas.

The development and widespread introduction of ONT-based sequencing is likely to accelerate in the next few years. It seems likely that many large diagnostic laboratories, and all diagnostic laboratories seem to be large by definition nowadays, will have some sequencing capacity and it will soon be taken for granted in the same way that the introduction of MALDI–TOF has been adopted.

The advantages of having ready access to this technology is dramatically illustrated by events of the last few weeks, involving adenovirus and monkeypox. With regard to adenovirus, at the time of writing approximately 400 cases of acute hepatitis in previously healthy children have been reported around the world, predominantly in the UK. So far, more than 20 children have required liver transplants and several have died. What is the causative agent? It does not appear to be any of the 'normal' hepatitis viruses. We will not consider here the surprisingly widespread point of view that it must be a COVID-19 vaccine, even if

The Oxford Nanopore technology is a small-scale sequencing platform that has enabled many laboratories especially in developing countries to perform their own rapid sequencing.

few if any of the affected children have had a COVID-19 vaccine. Of course, these cases of hepatitis in children may be post-infectious sequelae of SARS-CoV-2 infection as part of a whole group of multisystem inflammatory reactions in different systems.

## Adenovirus type 49

In the UK most attention has focused on adenovirus and in particular type 49 – is this justified? Adenovirus infections are common in children and typically cause mild respiratory symptoms, and several ingenious hypotheses have been proposed for how the pathogenesis of adenoviruses might have changed to cause hepatitis in otherwise healthy children. Some have suggested that the lack of exposure to many pathogens during lockdown due to COVID-19 has left children's immune systems naive to a greater or lesser extent, rendering them more susceptible to severe infection. Alternatively, it may be that as with other respiratory infections, relaxation of pandemic restrictions has led to a large wave of adenovirus infections allowing a hitherto rare outcome of infection to become prominent. However, as has been seen with other respiratory viruses, relaxation of pandemic restrictions could have led to a massive wave of adenovirus infections, allowing a rarer outcome of infection to be apparent. Yet another hypothesis is that past infection or co-infection (with SARS-CoV-2 or an alternative pathogen), or exposure to a toxin, drug, or an environmental factor, has altered the host response to adenovirus infection.

There is a need not just for careful case control studies, which have already started, but also for improved methods for the detection of adenovirus or possible novel pathogens. A rapid sequencing method that would detect the presence of adenovirus type 49 or exclude it would help resolve this problem rapidly.

The even more recent appearance of monkeypox in areas where it is not normally seen poses a different problem; here there is no doubt of the organism that we are trying to detect but not an obvious method of detecting it.

## Rapid detection

The need for a rapid method for the detection of a pathogen, whether novel or established pathogens like adenovirus and monkeypox direct from the clinical specimen is clear. Such a method must be 'agnostic', that is to say it shouldn't rely upon any *a priori* belief of the likely causative organism but should just detect and sequence what is there.

That means it's unlikely to be based upon a panel of primers to suspect pathogens, however widespread the coverage of the primers, because this requires an underlying assumption of what the organism of interest is and precludes the detection of any unsuspected or indeed novel pathogens. This really means that the method cannot be PCR-based but has to be sequence-based, and to not only work direct from the clinical specimen but provide results in a relatively short space of time. If the cost of the analysis is so high that specimens have to be batched, which is often the case with Illumina based sequencing, then a key advantage is lost.

This is of course a very demanding brief. Such a method also has to be able to detect both DNA- and RNA-based organisms in the presence of large amounts of human DNA, which provides competition for the sequencing reaction primers and reagents. The amount of human DNA in a specimen is likely to outnumber that of any pathogen by 1000-fold, perhaps even by a million or more. In an ideal world the method would also provide information about resistance markers and, if enough genome coverage is obtained, possibly allow subtyping of an organism (eg adenovirus).

This may seem impossibly optimistic but there are signs that it is in fact tantalisingly close, and Adela Alcolea-Medina, Clinical Scientist from the Centre for Infectious Diseases Research (CIDR), Guy's and St Thomas' Hospital, London, will be updating us on her research in this area. Many will remember the talk by Professor Jonathan Edgeworth, the Head of the CIDR unit, last year, which touched on this area.

## Quality control

The introduction of sequencing and other molecular technologies into diagnostic microbiology brings its own quality control problems, and Dr Elaine McCulloch, QCMD Glasgow, will be discussing the need for quality assurance in molecular diagnostics and sequencing.

## Resistance update

Although the focus of the meeting is primarily on the use and implementation of new technology, we are also aware of the huge number of other factors that are as much political as they are technological. Thus, Dr Katie Hopkins, Clinical Scientist Microbiology, UKHSA at Colindale, will be giving us an update on 'Current methods and problems in testing for antimicrobial resistance', which should prove invaluable for most attendees. We are therefore particularly pleased that Dr Esmita Charani, Research Lead (Practice, Design and Engineering), NIHR Health Protection Research Unit, Imperial College London, will be discussing antimicrobial stewardship from an international perspective.

Register to attend the 37th BSMT Annual Scientific Conference at https://www.bsmt.org.uk. The conference brings together biomedical scientists who work in microbiology and provides a comprehensive overview of current microbiological research and discoveries and is designed to encourage discussion and collaboration.