CULTURE VS MOLECULAR

Microbiologist **Dr Mark Wilks** looks at the key themes and messages that emerged from this year's British Society for Microbial Technology conference.

The recent 32nd Annual Scientific Conference of the British Society for Microbial Technology was entitled "Hot Topics in Microbiology". It focused on areas in medical microbiology where change has been rapid and is likely to be more even more so in the near future.

In diagnostic clinical virology, there has been a wholesale move from culture to polymerase chain reaction (PCR) and, more recently, sequencing. In contrast, diagnostic bacteriology has remained largely culture based. There are many reasons for this, including cost, the necessary skillset, and that the fact that culture methods, whatever limitations, are generally adequate.

Opinions have been polarised, with some refusing to engage with the new molecular methods, while others look down upon those still using culture and insist that PCR is now outdated and next generation sequencing (NGS) is the only way to go. Some have suggested that the only obstacle to high-throughput sequencing is the "innate conservatism of the profession".

The range of different topics covered at the meeting shows that there is, in fact, no conflict between the two different methods. Great gains are being made using molecular and cultural approaches to cope with different situations and, in some cases, a combined approach yields the best results. Let's look at how the approaches are being used in some rapidly changing fields.

Improving sepsis diagnosis

Professor Paul Dark, University of Manchester and NIHR Clinical Research Network Critical Care Lead, gave the first presentation on "Moving towards delivering precision medicine in sepsis".

Here we have the unusual situation where the gold standard – a positive blood culture, is actually not very good. Blood cultures are negative even in those in which sepsis is strongly suspected on

clinical grounds. The most important reason for this is probably prior antibiotic treatment, rather than the inadequacy of culture itself. As well as being insensitive, culture methods are often too slow to be useful in cases of severe sepsis.

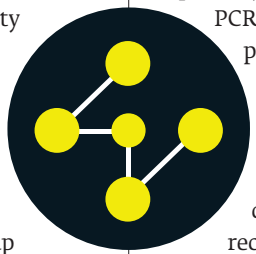
There are unlikely to be any significant improvement in culture methods, so what are the molecular alternatives? There are increasing efforts to develop molecular methods to rapidly detect bacterial and fungal DNA directly from blood without the need for blood culture. These are not affected by trial prior antibiotic treatment. Several CE marked methods were reviewed and showed great potential, although the costs of each test was high.

A complimentary molecular approach is to look at host biomarkers, such as CRP and PCT, released into the circulation in response to acute pro-inflammatory stimuli. Bacterial stimuli are associated with rapid and high responses and, crucially, they fall rapidly with correct treatment for bacterial infection. So, when used quantitatively, they have the potential to aid antibiotic initiation and discontinuation decisions. Although of course they don't give any direct information about causative pathogen or antibiotic susceptibility.

It's likely that a combination of molecular methods to detect DNA and host biomarkers will give the best results and lead to major advances in the reliable and rapid diagnosis of sepsis.

Aetiology of community acquired pneumonia (CAP)

One area in which the superiority of molecular methods has clearly been shown is in determining the aetiology of community-acquired pneumonia. In the pre-antibiotic era, *Streptococcus pneumoniae* could be isolated in up to 95% of cases, but in the majority of cases the credible pathogen is not isolated. It's not clear whether this represents a genuine change in the aetiology of the disease, more widespread use of antibiotics or both.



Dr Kate Templeton, Consultant Clinical Scientist, Edinburgh, described a recent landmark study in which they performed quantitative multipathogen testing of sputum samples in adults hospitalised with CAP. They collected mucopurulent sputum samples (96%) and endotracheal aspirates (4%) from 323 adults with radiologically confirmed pneumonia admitted to two tertiary care hospitals in the UK. They performed quantitative culture and multiplex real-time polymerase chain reaction (PCR) for 26 respiratory bacteria and viruses. With PCR, they identified a potential pathogen (bacterial or viral) in 87% of patients, compared with 39% using culture alone. Predictably, PCR detected bacteria more frequently than culture in patients who had received antibiotics (77.6% vs 32.1%).

Of course the isolation of a credible pathogen does not prove that it was responsible for the disease in every case, especially as many of the bacteria detected are carried in the upper respiratory tract without apparent harm in much of the population. Nevertheless, the results of the study are hugely encouraging.

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Identification of Mycobacteria

NGS to detect *Mycobacterium tuberculosis* from sputum samples has been shown to be possible, however, at present the detection of mycobacteria relies on culture. Dr Pieter Jan Ceyssens, Head of the Antibiotics and Resistance Unit at the National Reference Centre for Mycobacteria and Tuberculosis in Belgium, described the use of MALDI-TOF for the rapid identification of mycobacteria. In this case, the molecules are proteins and not nucleic acids, as in the other examples described.

The identification of cultured bacteria by MALDI-TOF has revolutionised the way of working in most labs in the UK and Europe. The vast majority of bacteria and yeasts can now be identified in minutes with minimal preparation. However, mycobacteria and filamentous fungi have proved much harder to identify. Dr Ceyssens described some simple methods in which positive cultures are heat killed, extracted with ethanol, sonicated and then loaded onto the MALDI-TOF in the usual way. This has allowed the identification of approximately 90% of the different species of non-tuberculosis mycobacteria that are encountered in clinical laboratories in a matter of minutes, a huge step forward over existing techniques, which are complex, expensive and take several days. This cheap and rapid molecular method may turn out to be superior to NGS in the majority of cases.

Microbial dark matter

Professor William Wade, from Queen Mary University of London, showed how molecular and cultural methods can be used in conjunction to greatly increase our knowledge of microbiology in a field where the limitations of culture have perhaps been too easily accepted. His talk was worryingly entitled "Cultivating the Uncultured". This turned out not to be a reference to the audience, but to an extremely ingenious and painstaking approach to growing new bacteria from the mouth.

Bacteria and yeasts can now be identified in minutes with minimal preparation

Oral bacteria are typically fastidious and slow growing – requiring complex media and long incubation times. Many are strict anaerobes requiring extra care in sample collection, transport and incubation. A comprehensive cultural analysis of samples is difficult, meaning that it is only possible to analyse small numbers of specimens and around half of oral bacteria detected by molecular methods are uncultivable.

Some of these belong to existing well characterised phyla, such as the Bacteroidetes, where there are many cultured representatives, such as *Bacteroides fragilis*, which have been known for over a century. Others constitute newly-discovered deep branching lineages with no cultivable representatives. The reasons for the lack of success could include under sampling - because culture is much more labour-intensive than molecular methods – and dependence on other bacteria in the community. This could be due to particular nutritional or signalling requirements, which are hard to reproduce in the laboratory or the bacteria may themselves be intracellular and parasitic and hence difficult to grow in pure culture.

His talk focused on his attempts to culture uncultivated members of the phylum *Synergistetes*. The underlying hypothesis was that some uncultivated oral bacteria required the presence of other bacteria, so it might be possible to grow them initially in mixed culture *in vitro*, with the aim of eventually weaning them off their dependence on other bacteria and thus get pure cultures.

The sample obtained from the periodontal pocket was cultured on blood agar and incubated anaerobically for 10 days. Plates were then photographed, replica plated and blotted onto nylon membranes. The membranes were hybridised with *Synergistetes* probe allowing the area of *Synergistetes* colonies to be rapidly located on the original plate. These colonies could then be subcultured on blood agar again and so the primary culture gradually enriched for *Synergistetes*. After eight passages, the mixture consisted of four well-described bacteria.

Molecular methods showed that there was not just the four species of bacteria, but the *Synergistetes* type Wo 90. By passage 12, this organism was able to grow independently, although next to a culture of *Parvimonas micra*. The organism was described, named (*Fretibacterium fastidiosum*) and its whole genome sequenced.

Remember that each passage took 10 days and 12 passages were needed to get the isolate in pure culture, so nearly six months of painstaking work were needed to recover the organism in pure culture.

This could only have been done by combining traditional cultural methods with molecular methods and it really shows the absurdity of trying to pose cultural and molecular methods as mutually antagonistic. BMS

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