
“It could be argued that the introduction of any new test should give as much weight to how the result will be handled as to the test itself”

Microbiologist **Mark Wilks** looks at some of the themes of the recent conference of the British Society for Microbial Technology.

TESTING TIMES FOR MICROBIAL TECHNOLOGY

A striking feature of this year's British Society for Microbial Technology (BSMT) conference was the increasing disconnect between traditional culture-based methods, which are still the mainstay of nearly all diagnostic microbiology labs for diagnosing infection, and the number of commercial molecular tests available.

There is now a huge range of what can almost be called "traditional" multiplex PCR tests, available in a bewildering number of combinations and run on a number of different platforms. For example, should you wish to introduce a commercial CE marked test for respiratory viruses into your laboratory, there are at least 20 available tests in various combinations of viruses and they only have Flu A and Flu B in common.

Having decided the particular combination of targets that suits your purpose (or budget), which one should you choose? In practice, it's impossible to do a trial of more than two or three for reasons of cost and time, so in practice the decision to introduce one test or another may be quite arbitrary and dependent upon the willingness of a particular diagnostic company to offer kits for evaluation at a reduced price.

Although rarely stated, with unusual pathogens it is virtually impossible to validate the claimed performance of a test. Raiding the back of your -80 freezer for elusive aliquots of a specimen which may have been frozen and thawed repeatedly is not ideal but is quite common.

Testing times

It would make more sense if there was some kind of coordination of testing perhaps, by a consortium of companies and laboratories which would make testing more rational and meaningful by specifying and supporting validation studies more precisely although, admittedly, the chances of this being done are very low. Ironically, the HPA evaluation service, which operated in this area, was disbanded 10 years ago, just as the rush of molecular tests was starting. In the absence of such a body, the onus is on manufacturers and distributors to support more ambitious clinical evaluation studies.

At the same time that more syndromic and multiplex PCR tests are being introduced, there is also a trend for much simpler tests designed for point-of-care testing (POCT) or near-patient testing (NPT). These are generally for single targets – for example, in the form of LFDs – and are being introduced at a relatively low cost. In fact, to call them simple is a bit of a misnomer, as to get a test to the level where it appears to be so simple to perform represents a huge technical feat, much more so than the conventional PCR test. Validation of these is generally simpler, as they have fewer targets but they bring their own problems of quality control when used outside the laboratory by staff who are not trained in testing.

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Next-generation sequencing

Another category, which is likely to be increasingly popular and on which increasing attention will be focused, is next-generation sequencing. Although still prohibitively expensive costs are decreasing – albeit not as rapidly as its proponents claim. To be affordable, in many cases, it is essential to batch specimens and to run them on the sequencer when a sufficient number of specimens has been accumulated to fill the capacity. This can often negate one of the claims of molecular methods over culture – that of speed.

In a few cases, most noticeably sequencing of *Mycobacteria tuberculosis* as described at the BSMT meeting has been astonishingly successful both for the identification of mycobacteria, studying its epidemiology and predicting its susceptibility to different antibiotics. It's worth bearing in mind that the success has been achieved by investing enormous amounts of effort and money in an area where the number of isolates is relatively low, traditional methods, although reliable, have been often very slow, and the need for accurate ID and sensitivity is paramount. Having said that, the basis of some of the costs showing that molecular methods are cheaper than conventional methods seem quite dubious to me.

When it comes to applying next-generation sequencing directly to clinical specimens, as opposed to pure cultures, the problems of interpreting results increase massively. It seems unlikely that it will be possible to overcome these within the next three to five years sufficiently for the methods to be applied in routine diagnostic laboratories. Most reagents are contaminated with low levels of bacterial DNA and these are often detected when sequencing sites of low microbial load. There is very little data on reproducibility of the results, for example how reproducible are successive runs from the same clinical specimen.

This kind of experiment, although simple in principle, is quite expensive to perform and is rarely done, but information on reproducibility is essential if the technology is to be introduced into the clinical laboratory. A recent QC exercise in human genetics, where the technology is much more developed, showed that four laboratories using different platforms detected less than 80% of the gene targets in all the cases. As well as detecting a target, the problem of quantitation has to be resolved. What is the significance of a particular number of reads of a bacteria in a respiratory site where you might expect colonisation anyway? Answering that kind of question will take a considerable amount of time.

Host response

A relatively new area of interest is to look at the host response and use this in combination with a microbiology result to decide if treatment is warranted. The "traditional" markers of host response, such as C-reactive protein (CRP) and procalcitonin (PCT), have been in existence for a many years, but the significance (for example, of a positive CRP result to guide therapy) is still debated.

The belief in these tests seems, in some cases, to follow national preferences, which is never the sign of a good test!

The PCT test, which is more recent, appears more promising, especially when used quantitatively and serially to look for falls in levels over successive days as a guide to treatment. The fact that these tests been around for such a long time without having established themselves unequivocally suggests they probably never will, although there are large-scale trials in progress. Dr Kate Templeton, in her talk at the BSMT conference, looked at some of the more common current approaches, which is to look at multiple host response factors, rather than look at single markers, such as CRP or PCT.

A recent study showed that patients infected with influenza A showed marked differences if they were symptomatic or asymptomatic in their immunological response. Another study looked at host gene transcription or profiles, which appear to differentiate viral from bacterial pneumonia.

Patient management

Lastly, it should be noted that in many cases much attention is focused on the actual performance of the test and less regard is given to its clinical relevance. With the introduction of any test, however technically ingenious, sensitive and whether the result comes from a tiny LFD and or is the product of large and expensive molecular analyser, the question "will it make a difference to patient management" is too often ignored. It could be argued that as much attention should be paid to reporting results in such a way as to draw the attention of the relevant clinician and force them to act by stopping or starting antibiotics, isolation or whatever is necessary.

Nearly half a century ago a famous American microbiologist, John Bartlett, made the point that a result that was not conveyed to the ward by 11am when the physician made his or her rounds would make no difference to the management of the patient that day. This is something that has arguably become even worse, with the majority of lab results passively relayed electronically and whilst nominally available to all 24hours per day, in practice are often ignored. It could be argued that the introduction of any new test should give as much weight to how the result will be handled as to the test itself. BMS

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