

How analytical science contributes to rapid microbiological assessment

A recent meeting discussed technical and regulatory issues surrounding the use of rapid microbiological methods and the substantial advantages they offer to the pharmaceutical industry for product quality control and process analytical technology. **Joe Chamberlain** reports

The pharmaceutical industry is facing unprecedented pressures, said Paul Newby, GlaxoSmithKline, Barnard Castle, County Durham, with research and development costs increasing year on year and reduced profits from fewer blockbusters, reduced exclusivity periods and increased patent challenges. Possible relief from these pressures will come from process optimisation by way of process analytical technology (PAT). Rapid microbiological methods (RMMs) have a significant part to play in achieving PAT objectives. Some barriers to the introduction of RMMs are technical ones. Many test systems are designed for different industrial sectors and there may be lack of understanding of pharmaceutical sector requirements. Guidance for the new user may be unclear and suitable suppliers with an understanding of good manufacturing practice and validation requirements may be lacking. Other barriers may be cultural and organisational. The pharmaceutical industry is a highly conservative one with a reluctance to change from compendial methods. The industry is uncertain of regulatory acceptance. There is often a lack of managerial commitment because managers may see no clear business requirement and have a perception that current traditional microbiological methods are simple and inexpensive.

These barriers may be overcome by considering RMMs as part of a wider design for the release of finished goods under PAT. It is important to spread the knowledge within the company, convince local management and the regulatory affairs department and build an effective business case. Significant resources, it must be recognised, are required for development and implementation. There must be a clear view of what is required and there must be close collaboration with suppliers and regulators, being realistic about implementation and the benefit to be expected.

RMMs are needed because current microbiological methods are potential bottlenecks to product release and cannot deliver real-time results. Dr Newby explained that GSK is interested in RMMs for product testing and

in-process control optimisation and the main technological contenders in this area are adenosine triphosphate (ATP) bioluminescence and solid-phase laser cytometry. The GSK implementation strategy is based on the important document from the Parenteral Drug Association: PDA Technical Report No 33, "Evaluation, validation and implementation of new microbiological testing methods". This report is intended to provide a general approach to the introduction of new microbiology methods in a government-regulated environment. It is also intended to provide guidance for the successful evaluation, validation and implementation of new microbiological methods needed by the pharmaceutical, biotechnology and medical device industries to assure product quality and provides a valuable guide to those developing and introducing RMMs into the product cycle.

Genotypic and phenotypic methods

Jim Bruce, Accugenix, Newark, Delaware, presented his contribution by satellite link. As long ago as 1980, Fox *et al* (*Science* 1980;209:457–63) proposed a natural system of classification based on phylogenetics rather than taxonomic characters. In many cases taxonomic characters are not phylogenetically valid; that is, morphological characters such as cell shape, mode of cell division and lack of cell wall can be misleading. Phylogenetic taxonomy is a universal system because all microbes have 16S RNA genes. No prior knowledge of identity is required and it is reproducible from laboratory to laboratory and over time. DNA sequences do not change according to how the organism was cultured or who is performing the test. Data are easily shared, transported and included in a database. It is more informative than phenotypic identification and there are no specific growth requirements.

A study was undertaken to determine species level accuracy and reproducibility using known ATCC (American Type Culture Collection)-type strains of organisms typically found in the pharmaceutical manufacturing environment. Eighteen ATCC strains were run in triplicate. Accuracy was evaluated according to whether the correct species was identified and reproducibility was evaluated according to how often the same result was achieved.

Genotypic methods were assessed using the MicroSeq microbial identification system from Applied Biosystems and the iBoPrinter microbial characterisation system from DuPont

Qualicon. Phenotypic methods were assessed using the Sherlock microbial identification system from MIDI (identification based on patterns of cellular fatty acids), the MicroLog MicroStation from Biolog (identification based on carbon use), and the Vitek apparatus from bioMérieux (identification based on substrate use). Sample data were interpreted according to the manufacturers' published guidelines. The study found that genotypic systems are more accurate and reproducible than phenotypic systems. 16S DNA sequencing provides phylogenetic information even when species identification cannot be made. Despite the often high capital cost, the effective price (cost per identification) of DNA sequencing is comparable with other systems. Outsourcing the microbial identification function of a pharmaceutical laboratory may provide more benefit at a lower cost with rapid results, concluded Dr Bruce.

ATP bioluminescence

ATP is considered an indicative component of living cells and its presence can be demonstrated by its participation in an enzyme reaction resulting in the production of light energy. Dr Newby delivered a paper by Christopher Randell, Wyeth Pharmaceuticals, Havant, Hampshire, on a detailed review of the use of ATP bioluminescence as a rapid biological method, including a survey of the main commercial instruments: Rapiscreen from Celcis, MicroStar from Millipore, and Pallchek from Pall/Gelman. The method is primarily qualitative and acts as a screening process for products or for environmental samples. It uses test protocols similar to the conventional methods and any growth can be identified for information. The assay generally reduces laboratory time and increases flexibility. This RMM encourages inventory savings, with material spending less time on storage in the warehouse. Although primarily used in the cosmetic and toiletries industry, it has been shown to be a useful technology in the pharmaceutical industry and has gained regulatory approval. However, Dr Newby said, there are initial investment and servicing costs, running costs can be higher than with traditional methods and sensitivity can be too good.

Laser-scanning cytometry

Robert Johnson, Pliva Pharmaceuticals, Petersfield, Hampshire, described the operation of the ChemScanRDI from Chemunex

Details The meeting, organised by the **Joint Pharmaceutical Analysis Group**, took place at the Royal Pharmaceutical Society's London headquarters on 18 March. Dr Joe Chamberlain is a former editor of *The Journal of Pharmacy and Pharmacology*

which uses laser-scanning cytometry for rapid microbiological measurements. The analysis is carried out in three steps: membrane filtration, cell labelling and laser scanning. The data processing facility evaluates the signal according to colour, shape and light intensity. Using the system, results are available in two hours, with enhanced sensitivity (single cell detection is claimed). It detects bacteria, yeast, moulds and spores in a single test protocol, and the results are independent of culture conditions. There is good recovery of injured or stressed organisms and sample analysis is automated. However, samples must be filterable and sample preparation is labour-intensive. There is no facility to identify micro-organisms, although morphological attributes (rods, cocci, spores) can be discerned. Sample preparation limits the ability to recover micro-organisms for further evaluation. The method can be applied to routine analysis of pharmaceutical grade waters, sterility testing, environmental monitoring, material testing and in-process control.

A thorough evaluation of the equipment showed that the ChemScanRDI consistently met defined operational parameters. False positive results were minimal. Good reproducibility, accuracy, linearity and precision ensured the system could be used without microscope confirmation. For routine water monitoring, the ChemScanRDI provides better results than the standard plate count.

ChemScanRDI accurately and precisely detected different bacterial strains, bacterial spores, yeast and fungi within a mixed culture; equivalent or better results were obtained compared with those obtained by the standard plate method. The ChemScanRDI is a more sensitive technology for the detection of biofilms compared with current methods. ChemScanRDI is the only current technology with the sensitivity and near real-time results for supporting in-process monitoring.

Dr Johnson concluded that the ChemScanRDI would build quality into pharmaceutical products during processing and therefore supports the ideals of PAT and parametric release.

Technological progress

Stephen Denyer, University of Wales, Cardiff, reviewed progress in RMMs, recognising the term "rapid" was a relative one. Professor Denyer placed emphasis on the European perspective, performance characteristics and future developments.

Microbiological tests could be either for detection (qualitative tests for the presence or absence of micro-organisms, quantitative tests for the enumeration of micro-organisms) or for identification (classically biochemical and morphological characterisation, now incorporating compositional analysis). The tests themselves could be classified as growth-based methods (techniques offering early detection of growth, and media development to improve detection), direct measurement by

direct observation, or cell component analysis (either phenotypic characteristics or genotypic characteristics).

Professor Denyer foresaw that the important growth-based technologies would be those based on impedance or bioluminescence methods. Direct measurements would be based on solid-phase cytometry and flow cytometry. Phenotypic methods of cell component analysis would continue to be based on fatty acid profiles and biochemical assays based on physiological reactions. Genotypic methods would depend on nucleic acid amplification techniques, ribosomal rDNA amplification and sequencing, and automated genetic fingerprinting or ribotyping.

Future trends, said Professor Denyer, will involve immunoaffinity (magnetic) capture, advances in laser scanning for both DNA fluorescent probes and immunofluorescent probes, adenylate kinase use for ATP amplification, and real-time detection of products of polymer chain reactions.

A regulatory viewpoint

The Medicines and Healthcare products Regulatory Agency (MHRA) has actively encouraged the pharmaceutical industry over the past 15 years to investigate and implement rapid microbiological methods, said Paul Hargreaves, MHRA, London, for the reason that this improves patient safety. Industrial microbiologists are enthusiastic about RMMs and many submit superb validation protocols. The MHRA is more than willing to discuss RMMs, review validation protocols and discuss possible uses ranging from screening tests, bioburden and even sterility testing.

It is disappointing, therefore, that RMMs have not been introduced more widely. Mr Hargreaves made a special offer to the audience; to encourage a wider uptake, MHRA assessors and inspectors are inviting an interest group such as the Parenteral Society or the Parenteral Drug Association to submit a dummy application for MHRA assessment and feedback.

The MHRA is keen to assist in lowering or removing any perceived regulatory barriers. Influence can be used at the European level through the EMEA quality working party and the ad hoc GMP inspectors group. If communication is a barrier, then the decision makers in the company that the MHRA needs to communicate with need to be identified to eliminate a 15-year impasse, and the MHRA is willing to organise a meeting or conference specifically for the decision makers. The MHRA has excellent communication with the key people at the US Food and Drug Administration with regard to RMMs and the MHRA and the FDA can work together prior to a submission to smooth the way. There may well be some issues, but now is the ideal time with the momentum generated by the PAT initiative to work together to introduce RMMs to the advantage of the patient and to the industry.

There may be barriers with regard to the validation and acceptance of RMMs. There is

much less pressure to accept many of the new alternative methods. There appears to be less academic interest and few published papers that address pharmaceutical aspects. Extravagant and misleading claims by equipment manufacturers do much harm and can set back the acceptance of the technology by many years. It is important that the theoretical basis for the test be fully documented. It is not acceptable to rely upon the manufacturer's claims or advertising and these are not an adequate substitute for refereed papers in reputable journals. Articles written by manufacturers in trade journals are also unlikely to be acceptable.

Transfer technology is another perceived barrier. It is not easy to transfer technology from the food and medical microbiology sector to the pharmaceutical industry. Whereas the food industry is looking for indicative numbers of micro-organisms and medical microbiology deals with large numbers of micro-organisms from swabs, the pharmaceutical industry requires new and different protocols and the specific problems, including equipment validation, validation of the technician and validation of the method must be addressed.

RMMs may play a significant role in improving the quality of medicinal products by enumerating and perhaps identifying micro-organisms that would previously have gone undetected. The introduction of RMM techniques for the counting and identification of micro-organisms in liquids and on surfaces may lead to radical changes to pharmaceutical microbiology. Control of biofilm in water systems may become the major challenge over the next few years.

Development of RMMs should be encouraged, said Mr Hargreaves, but their suitability will be subject to scientific assessment. RMMs will provide the means of enhancing patient safety, particularly for immunocompromised patients. The use of RMMs will require the employment of qualified microbiologists who are able to understand the basis of the techniques used, he concluded.

Equivalence

A panel discussion involving all the speakers followed the main presentations. A particular concern was the matter of demonstrating equivalence of the newly developed methods with the existing methods. It was emphasised that demonstration of equivalence meant demonstration that the new method was at least as good as the existing method for the purpose intended. It would be perverse to try to prove that the rapid method was as bad as the existing method.

In fact it was noted that, because of the increased sensitivity of the new methods, the FDA did not insist on demonstration of equivalence. This debate served to emphasise that the new methods being developed as rapid methods were, in fact, improving the overall analytical situation and thereby improved the quality and safety of the products.