Microbiology of normally sterile sites: enrich your culture

Dr Valerie Bevan reports on behalf of the British Society for Microbial Technology from its recent annual scientific meeting, held in London.

The British Society for Microbial Technology (BSMT) holds annual scientific symposia at Colindale, north London, and a regional symposium every two years. This report covers the most recent meeting, held in May. The symposium was well attended by over 140 delegates, and was supported by a comprehensive commercial exhibition.

Professor Eric Bolton chaired the morning session and Professor Brian Duerden chaired the afternoon session of this excellent meeting. The presentations stimulated wide-ranging discussion at the end of each session until time forced a halt, and feedback highlighted how much those who attended enjoyed the stimulating talks.

STANDARDS IN MICROBIOLOGICAL INVESTIGATIONS

As usual, the symposium attracted excellent speakers. The programme commenced with Dr Mat Donati, a consultant medical virologist at Public Health England (PHE) Microbiology Services, Bristol, and joint chair (with Ruhi Siddiqui) of the working group for developing syndromic algorithms (part of UK Standards for Microbiology Investigations), who provided a visionary and humorous introduction to the day by comparing conventional and molecular methods of diagnosis and highlighting the different applications of the newer techniques. Dr Donati stressed that we need a diagnostic approach that detects the most important pathogens, delivers results in a timely manner, uses the most appropriate technology and ensures good practice.

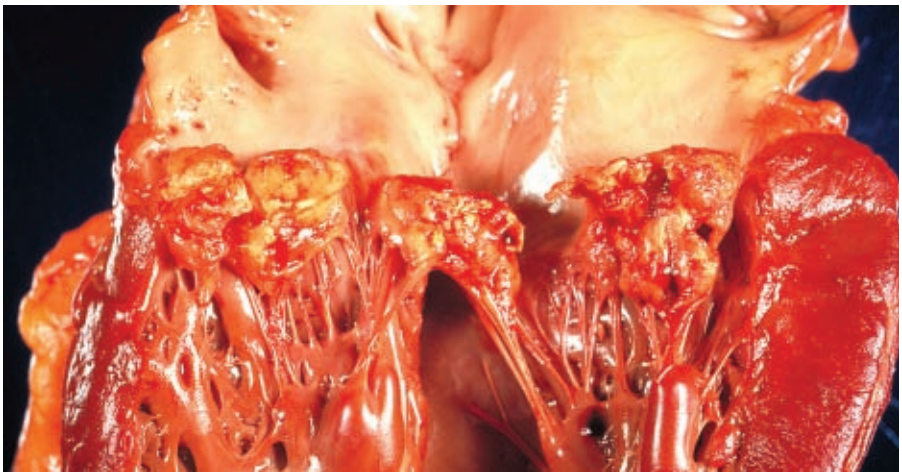
This approach provided the opportunity for Dr Donati to introduce the background and benefits of UK Standards in Microbiological Investigations (SMIs), which are a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology. A recent introduction is syndromic algorithms, which are the overarching guide to the more detailed investigations. Dr Donati stressed the increasing importance of the construction of SMIs relevant to the pace of modern diagnostics.

The UK SMIs are primarily intended as a general resource for practising professionals in the field of laboratory medicine in the UK. They recommend good standards (not minimum, not best), are not mandatory (but laboratories should be able to demonstrate equivalence, or better) and are useful as a clinical, laboratory, educational, electronic ordering and commissioning reference. They are also useful as a standard for audit and a benchmark against which to assess and improve methods.

Dr Donati described the process of the development of SMIs, stressing the importance of the support from professional organisations (including the IBMS) and the value of the opinions of users in contributing to the consultation process (UK SMIs can be accessed online at www.hpa.org.uk/smi). Dr Donati's final slide predicted a time when we would access a genome on a mobile phone.

16S, PAST, PRESENT AND FUTURE

Dr Derren Ready, lead clinical scientist at PHE Royal London Hospital, then gave a clear and illustrative talk entitled '16S, Past, Present and Future', which described the theory and practical applications of polymerase chain reaction (PCR)-based techniques of 16S ribosomal RNA (rRNA) gene sequencing. He discussed sequence matching, sample contamination and inhibition, and illustrated



CDC/Dr Edwin P Ewing Jr

Subacute bacterial endocarditis involving the mitral valve.

the use of the technique in cerebrospinal fluid (CSF) samples. Dr Ready went on to illustrate how multiplex real-time PCR techniques reduce time, increase the sensitivity of the test, and reduce costs, and are especially useful in low-yield sample types such as infective endocarditis.

A case study was presented of a 14-year-old girl who had been to the Middle East two years previously. She presented with a complex history and underwent multiple investigations which identified spleen abscesses on ultrasound and numerous negative blood cultures. Using 16S PCR, *Brucella* species was detected and the patient was treated successfully, showing a good clinical response with regression of the splenic abscesses.

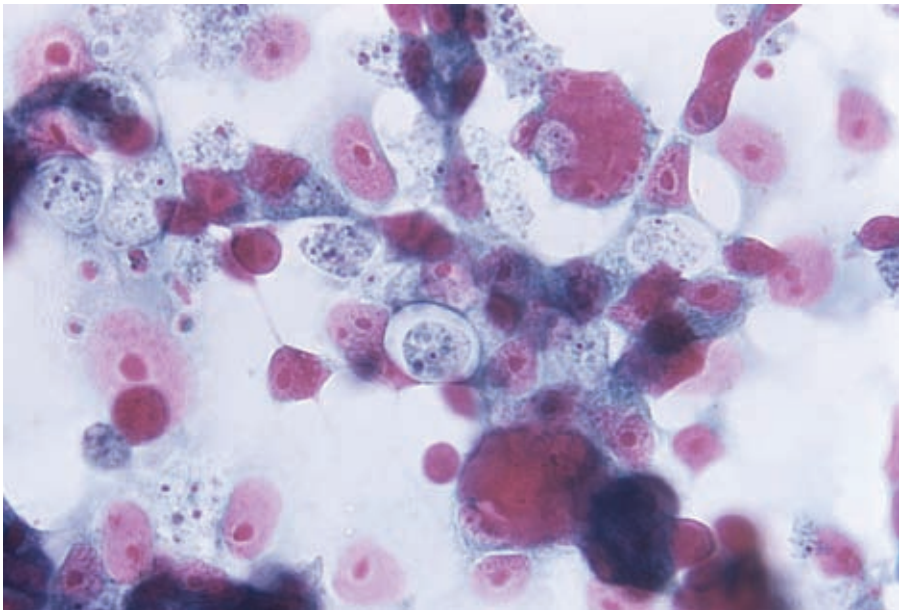
MALDI-TOF

The third presentation of the day was given by Dr Mark Wilks, lead clinical scientist at Barts Health NHS Trust, London, on mass spectrometry in diagnostic microbiology, concentrating on matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF). Dr Wilks described the theory, development and practical application over the past 20 years of 'MALDI' (which ionises molecules of a bacterial colony), followed by the role of the 'TOF' mass analyser (which measures the time it takes for ions to reach the detector). The commercially available MALDI-TOF systems were illustrated, and Dr Wilks emphasised the importance of having an adequate database. Furthermore, he stressed the importance of controls when he posed the question: "How do you know it's right?"

Examples of rapid identifications were given to show the benefits in blood cultures, including improving the treatment choices for patients, and in the identification of mycobacteria, fungi and viruses, compared with molecular methods and more conventional procedures. In summary, Dr Wilks' comprehensive presentation highlighted the dilemmas facing microbiologists using new techniques, in balancing the need for rapid identification and diagnosis with confidence in the accuracy of the results generated.

CLINICALLY IMPORTANT FREE-LIVING AMOEBAE

The last presentation of the morning session was given by Claire Rogers, head of the Teaching and Diagnostic Unit, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, on free-living amoebae in CSF and amoebic keratitis. Ms Rogers described the rare but often fatal infections that occur when free-living amoebae infect the central nervous system, and the sight-threatening keratitis caused by *Acanthamoeba* species. Symptoms, methods of sample investigation by microscopy, culture and/or molecular methods, and treatment were described for



CDC/Dr George R Healy

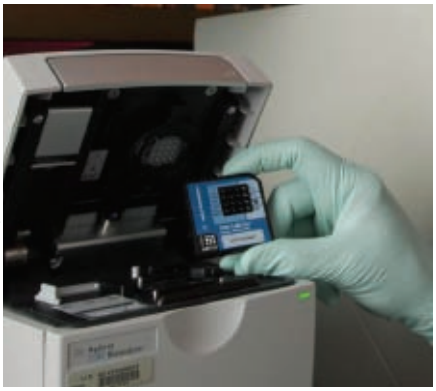
Free-living amoebae in a human brain tissue specimen, in a case of primary amoebic meningoencephalitis (trichrome stain, original magnification x500).

Naegleria fowleri, *Balamuthia mandrillaris* and *Acanthamoeba* species. Judging by the feedback received, this area of microbiology was new to many members of the audience, and it indicated how well Ms Rogers' presentation had been received.

RAPID DIAGNOSIS OF BACTERIAL SEPSIS

The first presentation of the afternoon session was a thought-provoking exposition from Dr Vanya Gant, Divisional Clinical Director for Infection, University College London Hospital NHS Foundation Trust, who gave an entertaining and pertinent clinical view of the rapid diagnosis of bacterial sepsis. Dr Gant explained that there are 20 million cases per year of bacterial sepsis worldwide, leading to 135,000 deaths per year in Europe.

Dr Gant then described the impact of bloodstream infections in terms of days taken in intensive care, financial costs, the importance of good turnaround times and successful interventions. He then proposed various options for technology-driven solutions and provided a glimpse of a likely future where a 'lab on a chip' becomes possible. In response to his question: "So we have the technology but what are we going to do with it?", Dr Gant suggested that price was not the only issue. Despite the political requirement to increase productivity and services, he believes there is resistance to modernising microbiology from many quarters. However, Dr Gant remains determined to continue to promote the better, faster diagnostics that are available now, and those which are just around the corner.



CDC/CDC Connects, James Gathany

A microfluidic 'lab on a chip' – a technology-driven solution and a glimpse of the future of diagnostics.

BLOOD CULTURE DILEMMAS

Dr Peter Cowling, Director of Microbiology at Path Links, and chair of the working group for developing UK SMIs for clinical bacteriology, gave a topical and highly relevant presentation on the dilemmas that concern diagnostic laboratories over blood cultures. Dr Cowling pointed out that responsibility for blood cultures starts at the point the clinician considers the differential diagnosis, not when the specimen arrives in the laboratory.

Dr Cowling highlighted the dilemmas associated with producing rapid results from blood cultures. Microbiologists need to meet the needs and expectations of users for relevant and rapid treatment of patients with the current technology available and the need for new rapid molecular methods for the whole specimen pathway. Other pressures are the emergence of multiresistant organisms, the need to reduce blind treatment and the need for near-patient testing.

Acknowledging the contribution of Dr Shabnam Iyer, Dr Mike Weinbren and Mr Ian Sturgess, Dr Cowling discussed changes in the blood culture SMI, particularly in relation to setting standards in the revised document on turnaround times, pre-incubation advice, the management of specimen transport from distant sites that sets a contamination target of less than 3%.

'Currently, 98% of joint replacements are for hips, and an infection rate of 1% leads to a painful virulent infection'

The revised SMI includes recommendations on systemic inflammatory response syndrome (SIRS) and neonatal sepsis, and includes recommendations on direct sensitivity testing and molecular methodologies.

Dr Cowling stressed the importance of developing SMIs in collaboration with nominated representatives from professional organisations to ensure joint ownership of the methods. He also pointed out that the process for developing SMIs is accredited by the National Institute for Health and Care Excellence (NICE) and developed under the International Organization for Standardization (ISO) 9001:2008 quality standard which gives them additional authority.

MICROBIOLOGY OF PROSTHETIC JOINT INFECTIONS

Dr Robert Nelson, consultant medical microbiologist at Wrightington, Wigan and Leigh NHS Foundation Trust, gave the final presentation of the day on the microbiology of orthopaedic joint infections. Dr Nelson started by paying tribute to Sir John Charnley, who was the pioneer of low-friction arthroplasty and established a unit at Wrightington in 1961. Sir John realised the risks of infection from contaminated air and pioneered ultraclean ventilation for operating theatres.

Currently, 98% of joint replacements are for hips, and an infection rate of 1% leads to a painful virulent infection. Early and late infections are usually caused by *Staphylococcus aureus* (sometimes meticillin-resistant and sometimes complicated by small colony variants), while delayed-onset infections take longer to manifest and are caused by less-pathogenic organisms such as coagulase-negative *Staphylococcus* species or *Propionibacterium* species.

Dr Nelson also described the biofilms that form on prostheses *in situ* and the difficulties of successful treatment. He also described preoperative precautions, how to take operative cultures and how many should be taken. He described the options for laboratory processing and how to interpret the culture results. Dr Nelson said that every isolate should be regarded as potentially significant and identified, with a full range of sensitivity tests undertaken. ■

The BSMT meeting was kindly supported by Alpha Laboratories, BD Diagnostics, BioConnections, bioMérieux UK, Bruker UK, Don Whitley Scientific, Instrumentation Laboratory, Launch Diagnostics UK, Mast Diagnostics, Medical Wire & Equipment (MWE), Oxoid (Thermo Scientific) and Pro-Lab Diagnostics.

Dr Valerie Bevan CSci FIBMS (vbevan@vbevan.co.uk) is London region IBMS Council member and BSMT chair.

The BSMT is a not-for-profit organisation composed of microbiologists working as healthcare scientists and medical microbiologists, mainly in the NHS and Public Health England (formerly the Health Protection Agency). The society's aim is to promote an exchange of information on laboratory practices in clinical microbiology, aimed particularly at bench microbiologists. If you would like to become more involved with the BSMT committee, please contact any committee member (email addresses may be found online at www.bsmt.org.uk).

The next BSMT regional symposium, on the Microbiology of the Compromised Host, will be held on 11 October at the Mercure Hotel, Sheffield. More information is available online (www.bsmt.org.uk).