Rapid detection of bacteraemia

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Bacteraemic sepsis has a high mortality that can be reduced by early diagnosis and initiation of appropriate antimicrobial therapy [1]. Rapid confirmation of the diagnosis and identification of the causal agent provide guidance on the adequacy and duration of antimicrobial therapy and on the need for source investigation. Clinical microbiology laboratories have rightly placed great emphasis on this aspect of their practice. As causal organisms are usually present in low titre, direct microscopy is impractical and laboratories have generally relied on culture of blood in broth, which is relatively insensitive and too slow to influence initial management. Phenotypic methods for the identification and antimicrobial susceptibility testing (AST) of isolates have progressively improved over the last two decades, but still require significant periods of incubation. Similarly, commercial blood culture systems have been refined with better systems for automated detection of growth in broth, but still require incubation for up to five days and subculture for the isolation of pathogens. Constantly monitored blood culture systems and automated identification/AST are now the norm in most clinical laboratories. Although there will undoubtedly be further development of phenotypic methods, with incremental improvements in sensitivity and time-to-detection, research and development now concentrated on molecular detection methods has the potential to result in a paradigm shift in our approach to the microbiological diagnosis of this condition.

Molecular methods applicable to the diagnosis of bloodstream infection can be divided into two broad categories: those employed to identify organisms (and resistance markers) in positive blood cultures and those that directly detect microbial DNA in blood after DNA extraction and PCR amplification. The former provides an incremental benefit by reducing time to identification, but is still dependent on current methods for growth in broth media. Assays can be further classified as single-pathogen specific, multiple-pathogen specific (targeting a variable number of common pathogens) and broad range. A great variety of in-house methods have been published using technologies ranging from *in-situ* hybridisation to micro-arrays and these have been reviewed elsewhere [2]. As development and validation of complex in-house methods is beyond the resources of most clinical laboratories, this discussion will concentrate on potentially useful commercially developed methods with multiple-species or broad-range detection.

Molecular methods have the potential to overcome many limitations of culture by providing rapid detection, quantitation and identification of culturable and non-culturable pathogens. Given that the time of initiation of appropriate antimicrobial therapy is crucial to patient outcome, the early use of initial broad spectrum antimicrobials will remain standard practice. However, rapid, specific diagnosis will allow the early narrowing or change of spectrum of antimicrobial cover and will obviate the need for further diagnostic investigation. Quantitation of bacteraemia correlates with severity of disease and outcome and is potentially useful for monitoring response to therapy [3]. Quantitative PCR has already shown utility for this purpose [4]. Some characteristics of an ideal system for detection of blood stream infection are shown in Figure 1.

Examples of commercially available systems for diagnosis of bacteraemia are shown in Table 1. A number of problems make assessment of new methods difficult. The current 'gold standard' is problematic as it is likely that blood cultures fail to detect about half of cases of septicaemia when clinical and other laboratory criteria are taken into account [5]. Prior antibiotics, sampling error, intracellular agents and non-culturable agents may all contribute to this problem. Detection of nucleic acid does not necessarily indicate the presence of viable organisms and, to complicate matters further, bacterial DNA is widely distributed in nature, necessitating the use of super-clean reagents. Extraction is a key step for nucleic-acid-based methods that must is some way deal with the high level of human DNA present in blood. A number of strategies for differential extraction and enrichment of bacterial nucleic acid have been used. Given frequently low organism loads, an efficient process is crucial. This is an area where

- ✔ direct examination of blood
- ✔ broad-range detection of potentially pathogenic bacteria and fungi
- ✔ able to quantify pathogen DNA
- ✔ high sensitivity and specificity for bloodstream infection
- ✔ detection of genetic markers for antimicrobial resistance
- ✔ unequivocal result interpretation
- ✔ minimal turnaround time
- ✔ highly automated

Figure 1. Characteristics of the ideal system for detection of bloodstream infection.

technological improvement is needed. Estimates of sensitivity and specificity of new methods compared with blood cultures are not included here but should be interpreted carefully in view of the above.

Protein-based methods such as Raman spectroscopy (based on vibrational fingerprints) and protein mass spectrometry are reliant on an initial culture step, which will always limit their sensitivity and dictate time to detection. PCR-based methods that target a limited number of high-frequency pathogens in whole blood can certainly reduce detection times for the majority of cases, although sensitivity will vary due to specific methodological factors and the number of targets included. Broad-range methods seem to hold the most promise for a comprehensive solution. The availability of rapid, low-cost sequencing makes methods based on analysis of 16S rDNA feasible, although it is limited to bacterial detection if no fungal target is included. A similar approach to fungal pathogens seems an obvious next step. Electrospray/ionisation mass spectrometry is a novel method that makes use of the ability to accurately measure the weight of PCR-generated fragments of four universally conserved bacterial housekeeping genes and three *Candida* rDNA targets. A computerised triangulation algorithm allows the accurate calculation of base composition, from which is inferred genus and (usually) species by database comparison. The method is also quantitative and this may aid both the interpretation of initial results and monitoring of response to treatment.

Significant problems remain to be solved before the ideal method is achieved. While a number of the nucleic-acid-based methods include markers for antimicrobial resistance, none addresses the issue of resistance detection in a comprehensive fashion. Even rapid, low-cost, whole-genome sequencing, which is now technically possible, seems unlikely to provide the answer in our current state of knowledge as correlation of genomic data with minimum inhibitory concentrations of antimicrobial agents is lacking. A number of the rapid methods available involve multiple steps and require considerable technical expertise to perform. Further automation and streamlining of data presentation and interpretation would greatly improve their utility. While rapid molecular methods hold the prospect of rapid and reliable diagnosis of bloodstream infection with the ability to improve clinical outcomes, the limitation of antimicrobial resistance prediction to the detection of specific genetic markers is a major limitation. This being so, it is likely that blood cultures will be with us for some time, although perhaps in parallel with or even guided by rapid molecular methods.

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Table 1. Examples of commercial systems applicable to rapid detection of bloodstream infection.

Product	Method	Specimen	Range	Quantitative	Resistance detection	Time to result	IVD♣ licence	Reference
Raman spectroscopy	Whole cell spectroscopy	Micro-colony	Broad	No	nil	21–23 hours*	?	6
UltraFlex 1 TOF/ MALDI Biotyper (Brucker Daltronics)	Protein mass spectrometry	Broth culture pellet	Broad	No	nil	16–17 hours*	EU CE-IVD	7-13
SeptiFast® (Roche)	Real-time PCR♦, hybridisation probes	Whole blood	25 species	No	nil	6–15 hours	EU CE-IVD	14-20
LOOXSTER®/ VYOO™ (SIRS-Lab)	Spin-column extraction, multiplex PCR♦ (gel)	Whole blood	40 species	No	4 resistance markers	8 hours	EU CE-IVD	21
SepsiTest® (MolZym)	16S rDNA PCR♦, sequencing	Whole blood	Broad	No	nil	4 hours	EU CE-IVD	22
Prove-it™ (Mobidiag)	PCR♦, micro-array	Blood culture broth	50 species	No	1 marker	18 hours*	EU CE-IVD	23
PLEX-ID BAC Spectrum (Abbott)	PCR♦, ESI Mass Spectrometry	Whole blood	Broad	Yes	3 resistance markers	4–6 hours	No	24-26

♣ *In-vitro* diagnostic device
* Assumes average 15 hours incubation to detection of significant blood culture growth
♦ Polymerase chain reaction

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Biography

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'Swabs' then and now: cotton to flocked nylon

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Microbiological sample collections using cotton-tipped swabs (with or without serum), Dacron™, rayon and calcium alginate, with shafts of wood, plastic and various thicknesses and types of metal have all been used over the years. The swabs have been contained in glass or plastic tubes with and without various types of transport media. Swabs are an easy and popular method of sample collection, although microbiology laboratories traditionally prefer tissue, body fluids or aspirates ahead of swabs. As microbiology laboratories increasingly adopt near patient testing and molecular detection methods to reduce test turnaround times, new sample collection methods are required to maximise the sensitivity of these expensive tests and reduce the possibility of failed tests due to sample inhibitors or poor collection techniques. Flocked nylon swabs have been developed by Copan in the last decade and are produced using a technique of spraying nylon fibres onto a rigid core. This has the effect of increasing the surface area for sample collection and also provides easy elution of the collected material. These swabs are polymerase chain reaction (PCR) inhibitor-free, RNase-negative and DNase-free and there is in addition a range of flocked swabs, specifically intended for forensic DNA investigations that are certified human DNA-free. The advantages, disadvantages and appropriate use of swab collections for microbial detection in the 21st century will be presented.

Sterile swabs are a popular and convenient method of collecting samples for microbiological analysis. A review of any standard microbiological textbook from the early decades of the 20th to the 21st century [1-7] provide lists of swab samples that are acceptable for analysis and some [2] have details of recommended swab types.

Swabs have always been considered inferior to fluid, aspirates or tissues but their low cost, convenience and ease of use have made them popular amongst clinical staff responsible for specimen collection.