Rapid detection of microbial infection in the ICU: an ongoing search for new diagnostic tools

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Abstract - Infectious diseases are common in patients admitted to the intensive care unit (ICU). Microbial culture and serology are traditionally used to establish the diagnosis, but take considerable time and may provide false-negative results after use of antibiotics or in the early phase of infection. Antigen detection, fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR) are increasingly used as alternatives for culture and serology in the routine diagnostic procedures of the microbiological laboratory. Advantages of these techniques are the short turnaround time to results, the higher sensitivity, and that these tests are presumably not affected by prior use of antibiotics. In addition there is a higher yield for detection of fastidious microorganisms and are very suitable for detection of one specific microorganism in samples with a high diversity of microbes. A disadvantage is that false-positive results may occur due to colonization or laboratory contamination. Furthermore, antimicrobial susceptibility data usually cannot be generated with these molecular techniques. This review discusses the pros and cons of these commonly used laboratory techniques for rapid diagnosis of infection in ICU patients.

Keywords - Infection, diagnosis, molecular detection, antigen detection test, fluorescence in situ hybridization, PCR, intensive care unit (ICU), critically ill.

Introduction
Infectious diseases are an important cause of morbidity and mortality in patients admitted to the intensive care unit (ICU). Approximately 20% of patients have an infection on admission to ICU and another 20% acquire infection during their stay there. [1-3]. Infectious complications are associated with excess mortality and a significant increase in cost and length of stay [3-5]. In practice, whenever infection is suspected, samples are obtained for microbiological investigations. Microbial culture and serological analysis are used for detection of microorganisms, but it takes some considerable time before results are available. As a consequence, clinicians may already have initiated or changed antimicrobial therapy based on clinical course and before results of microbial culture or serology are available. In addition, the yield of cultures is markedly reduced if samples are obtained after the initiation of antimicrobial therapy or if fastidious microorganisms are present. False-negative results can occur with serological analysis in the early phase of infection. Also, follow-up blood sampling may be required to interpret serological results.

Molecular diagnostic tests are increasingly being incorporated as a faster alternative to culture and serology in routine microbiological practice. Nowadays, most hospital laboratories are equipped to perform molecular tests under specialized conditions, supervised by a clinical molecular biologist and a medical microbiologist. The widespread introduction of molecular diagnostics has resulted in exciting new opportunities for the diagnosis and management of patients with infectious diseases. On the other hand, molecular tests have their own drawbacks that influence the usefulness of these techniques in routine practice. The strong advent of diagnostic techniques for rapid detection of infection necessitates a critical evaluation of the pros and cons of these relatively new techniques. In this review we discuss the potential use of molecular antigen detection tests, fluorescence in situ hybridization and polymerase chain reaction; currently the three most commonly used tests for rapid detection of microbial infections relevant to ICU patients.

Antigen detection tests (ADT)
Rapid detection of microbial antigens can be achieved with latex agglutination tests (LAT), immunochromatographic membrane tests (ICT), or enzyme-linked immunosorbent assays (ELISA). ADT are based on microbial antigens binding to specific antibodies. Visualization of antigen-antibody complexes is achieved by clumping of antibody-bound latex particles in LAT, formation of a line after immobilization of the complexes by capture on a nitrocellulose membrane (ICT), or a macroscopic colour change induced by antibody-bound enzymes (ELISA). These ADT provide results rapidly ranging from within 15 minutes to a few hours, are easy to perform and are not laborious. Another advantage is that some ADT do not necessarily require sampling at the site of infection, e.g. urinary ADT for Legionella pneumophila pneumonia. During infection, microbial components and antigens may leak and are transported into the bloodstream followed by excre-
tion in the urine. Testing of blood or urine with ADT is especially useful if sampling of the suspected site of infection is difficult or requires an invasive procedure. Important disadvantages of ADT are that false-positive results can be generated by colonization with a specific microorganism and that ADT results may remain positive for weeks after treatment [6-9]. This may result in a confusing situation if a patient develops another febrile episode without obvious cause and the clinician has to decide whether to treat for relapse of infection or to search for another cause of the fever. Also, vaccination against S. pneumoniae may result in positive ADT for a few days after administration of the vaccine [10]. Finally, cross-reaction with other microorganisms may lead to false-positive results in some tests, for example positive reactions were observed for some non-Aspergillus fungal species with the galactomannan assay and for urinary L. pneumophila ADT in a patient with pneumonia caused by Nocardia species [11,12]. However, this is uncommon and technical specificity of most ADT is high.

The most relevant ADT for ICU patients are those for detection of Streptococcus pneumoniae, L. pneumophila, influenza virus types A and B, respiratory syncytial virus (RSV), human metapneumovirus (hMPV) and Aspergillus species. These infections are usually community-acquired and related to the respiratory tract, with the exception of Aspergillus infection which is usually hospital-acquired. As such, ADT are most useful in ICU patients who present with community-acquired respiratory disease and in patients at risk for Aspergillus infection. Wide ranges of sensitivity for urinary ADT have been reported in the S. pneumoniae C polysaccharide cell wall antigen (54-87%) and for the serogroup 1 antigen of L. pneumophila (48-90%), in patients with community-acquired pneumonia (CAP) [13-16]. However, these studies only included small proportions of patients admitted to the ICU. Patients with more severe illness excrete higher concentrations of antigen and are thus more likely to have a positive ADT result [16-18]. As such, the highest sensitivity of these assays can be expected in critically ill patients. This is confirmed by one large study on sensitivity (72%) and specificity (90%), of urinary ADT for pneumococcal origin of CAP in ICU patients [19]. ADT for viral pathogens are usually performed on respiratory tract samples instead of urine or blood. The sensitivity of ADT to influenza virus types A and B has been reported to be 42-76% compared with viral culture as the reference standard in adult patients with respiratory tract disease [20-21]. These rapid tests for detection of influenza antigen enhance the quick clinical diagnosis of influenza and lead to reduction in antibiotic usage in hospitalized patients at low risk of concomitant bacterial infection [22,23]. ADT for RSV and hMPV have mainly been evaluated in respiratory tract samples from children with respiratory tract disease. Sensitivity of ADT was higher in detection of RSV (65-94%) than in MPV (63%-73%) [24-29]. To our knowledge, a thorough evaluation of ADT for influenza virus, RSV and hMPV has not yet been performed specifically in ICU patients; the performance in critically ill patients may be better than in general hospital or outpatient populations, similar to the performance of S. pneumoniae ADT. Finally, detection of galactomannan, an exo-antigen released by Aspergillus hyphae during growth, may be a useful tool for diagnosis and monitoring of invasive Aspergillus infection in ICU patients [30,31]. Although galactomannan ADT is a rapid

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**Table 1. Advantages and disadvantages of microbial detection methods discussed in this review**

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>CULTURE</th>
<th>SEROLOGY</th>
<th>ADT</th>
<th>FISH</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to identification</td>
<td>24-48 hours</td>
<td>1-4 hours</td>
<td>&lt;1 hour</td>
<td>1-4 hours</td>
<td>1-4 hours</td>
</tr>
<tr>
<td>Microbial detection¹</td>
<td>Universal/specific</td>
<td>Specific</td>
<td>Specific</td>
<td>Universal/specific</td>
<td>Universal/specific</td>
</tr>
<tr>
<td>Target of detection</td>
<td>Living microorganisms</td>
<td>Circulating antibodies</td>
<td>Circulating antigens</td>
<td>Living microorganisms</td>
<td>DNA/RNA of living and dead microorganisms</td>
</tr>
<tr>
<td>Detection of fastidious microorganisms</td>
<td>Difficult/slow</td>
<td>Possible</td>
<td>Not available</td>
<td>Difficult</td>
<td>Easy/rapid</td>
</tr>
<tr>
<td>Affected by antibiotics</td>
<td>Yes</td>
<td>Usually not</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Quantification of microbial load</td>
<td>Uncommon</td>
<td>No</td>
<td>No</td>
<td>Uncommon</td>
<td>Yes</td>
</tr>
<tr>
<td>Antimicrobial susceptibility Determination</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Risk of laboratory contamination</td>
<td>Low/moderate</td>
<td>Low</td>
<td>Low</td>
<td>Low/moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Laborious²</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Cost³</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

ADT, antigen detection test; FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction.
¹universal detection only applies for bacteria and fungi; virus detection includes a specific virus.
²increasing number of (+) indicates more/higher labour and/or costs.
³Cost of FISH and PCR is lower when more tests are performed simultaneously.
test, adequate clinical interpretation of results requires more time. For good interpretation, it is necessary to monitor galactomannan levels over time, because of the likelihood of false-positive results due to colonization, gastro-intestinal translocation, cross-reaction with substances in antibiotics (like piperacillin) or with other microbes, and laboratory contamination, e.g. with cardboard particles [7,32]. A recent meta-analysis shows that surveillance of galactomannan in serum has a mean sensitivity of 71% for proven cases of Aspergillus infection and of 61% for probable cases [33]. Some reports suggest that serum changes of galactomannan antigen precede radiographic changes and culture isolation of Aspergillus by several days, but other studies do not show additional value to standard clinical, radiographic and microbiological findings [31,33-35]. The value of routine galactomannan determination in relation to routine clinical practice needs to be further elucidated.

Fluorescence in situ hybridization (FISH)

The FISH technique is based on specific binding of probes to target ribosomal RNA followed by microscopical detection of fluorescence (Figure 1). A probe consists of a synthetic oligonucleotide or peptide nucleic acid (PNA) strand with a fluorescent label. The strand is complementary to the ribosomal RNA (rRNA) of a specific microbial species, genus or family and will hybridize with the ribosome at a specific temperature. Visualization of fluorescence by microscopy is achieved by the mounted fluorescence of probes bound to the high number of ribosomes in microbial cells. As such, FISH can only be used to identify intact, viable microorganisms. The analytical sensitivity and specificity of individual probes for target rRNA is high and aspecific binding of probe is uncommon [36]. Clinical sensitivity of FISH is related to the volume of sample that is tested and to whether the number of microorganisms is high enough to be recognized with microscopy. The microbial load involved in a certain condition determines whether FISH can be performed directly on a sample (CSF, sputum) or first requires culture (blood) to prevent sampling errors [37-39]. The major advantage of FISH is the short time to identification: 1-4 hours dependent on the probes and procedure used [40]. In a recent study at the VU University Medical Center we showed a considerable time-gain for FISH compared with standard microbial culture for identification of microorganisms in growth-positive blood cultures [36]. Other studies show that the majority of pathogens involved in meningitis and necrotizing fasciitis can also be identified within a few hours [37,41]. Another advantage of FISH is that mixed infections can easily be identified, for example with Staphylococcus aureus and coagulase-
negative staphylococci or different serogroups of Legionella species [36,42]. Also, samples with a diversity of commensal and potentially pathogenic microbial species can easily be analyzed, as was shown in respiratory tract samples of patients with cystic fibrosis [38]. Surprisingly, to our knowledge FISH has not yet been evaluated on sputum samples of patients suspected of ventilator-associated pneumonia despite the potential clinical benefit of a rapid diagnosis and the commercial availability of probes for the majority of pathogens involved in this condition.

A wide variety of FISH probes are commercially available, but for some microorganisms e.g. Streptococcus mitis or Enterobacter cloacae, no species-specific probes exist. Inclusion of a genus-, family-specific or eubacterial probe in the FISH test ensures that no false-negative results are obtained in such cases. Another disadvantage of FISH is that it is unable to discriminate between species with very homologous rRNA, for example between Escherichia coli and Shigella species [36]. Finally, FISH does not provide data on antimicrobial susceptibility. Therefore, adjustments of therapy based on FISH identification are empirical and based on general antimicrobial surveillance data.

Routine implementation of FISH would be most useful in conditions where rapid diagnosis is required and where identification leads to a change of therapy without the need for testing for antimicrobial susceptibility. Two recent studies in the USA show that FISH for discrimination between Candida albicans and non-albicans Candida species in positive blood cultures results in a significant reduction of caspofungin usage and saves $1800 per patient [43,44]. A retrospective study shows a reduction of length of hospitalization and use of vancomycin when FISH is used to discriminate between S. aureus and coagulase-negative staphylococci in blood cultures in non-ICU patients [45]. It is questionable whether using FISH on positive blood cultures will have much influence on antimicrobial management of ICU patients, because the time required for growth in blood culture may be too long and initial empirical therapy may already have been changed on clinical grounds before FISH results are available [46]. The cost-effectiveness of FISH identification in these studies is based on a clear switch from initial empirical therapy to microorganism-specific treatment. Despite the influence of setting and first choice of antimicrobial therapy on cost-effectiveness, these studies yield promising data on the benefit of FISH identification in specific conditions. Similar studies on routine use of FISH for other diagnoses are highly warranted, especially in patients with suspected meningitis or ventilator-associated pneumonia.

**PCR detection and quantification of microorganisms**

The principle of PCR is the specific multiplication of microbial DNA followed by detection of the amplification product. This is achieved with primers and probes: oligonucleotides with a short sequence that is complementary to target DNA. The probe oligonucleotide is bound to a fluorescent label (fluorescent dye with a quencher). Binding of probe to complementary DNA is followed by separation of the quencher from the fluorescent dye. This results in measurable fluorescence; the intensity of fluorescence correlates to the amount of PCR product. Monitoring of fluorescence intensity during the amplification process (so-called ‘real-time PCR’) enables quantification of the DNA in the sample.

The short turnaround time is an important advantage of PCR assays and makes them especially useful in serious conditions when a rapid diagnosis is required or in slow-growing microorganisms where culture requires many days. Another important advantage of PCR is its high sensitivity (1-50 microbes/reaction), which is especially useful in fastidious microbes. Also, PCR can easily be used to detect one specific microorganism in samples containing a high diversity of microbes. PCR assays are presumably unaffected by prior use of antibiotics and can add to the diagnosis in patients receiving antimicrobial therapy [47,48]. While bacterial growth is effectively inhibited by antibiotics in hours to days, the decrease in DNA of both living and dead bacteria will be much slower (days to weeks) [49,50]. This enables PCR to be used as a tool for the detection and monitoring of DNA under treatment. Finally, with real-time PCR, DNA can be quantified. Quantification and monitoring of DNA in patients with infection adds an important dimension to the qualitative or semi-quantitative results obtained with conventional diagnostics.

A disadvantage of PCR is that the choice of PCR primers and probes determines which specific microorganism can be detected. In addition to PCR assays for detection of a specific microbial species or genus, eubacterial or panfungal PCR reactions can be performed to screen for presence of any bacteria or fungi. These eubacterial PCR assays can only be done on normally sterile samples such as blood, pleural fluid and cerebrospinal fluid, because otherwise colonizing microbes will interfere with PCR and disturb subsequent sequence analysis for identification. Inhibitory components in tissue, e.g. haemoglobin and leukocytic DNA, can interfere with PCR efficiency and negatively affect PCR results [51,52]. Therefore, it is essential to include an adequate control reaction for detection of inhibition in diagnostic PCR assays. A ‘panviral’ PCR does not exist, because the genome of viruses is too diverse and dynamic, but virus group-specific assays can be used. The development of ‘PCR panels’ with a panel of primers and probes is needed, for example a ‘respiratory tract panel’ that includes PCR sets for the most prevalent bacteria and viruses involved in respiratory tract infection [53,54]. Another disadvantage of PCR is that the possibility of molecular susceptibility testing is still very limited, and only available in a few specific cases such as for determination of methicillin resistance in S. aureus and for isoniazid and rifampicin resistance genes for Mycobacterium tuberculosis. Finally, environmental contamination with DNA can easily occur and the combination with such a highly sensitive technique makes PCR more prone to laboratory contamination than conventional techniques [55].

Because of their short turnaround time, PCR assays can be useful for the rapid diagnosis of invasive infection. McMullan and colleagues show that twice-weekly monitoring of blood samples by PCR enables the early detection of candidaemia (sensitivity 91%, specificity 100%) in ICU patients and the discrimination between Candida species that are usually sensitive for fluconazole treatment and those that are frequently resistant [56]. Also, PCR assays can be used to detect S. aureus or Enterococcus...
faecalis DNA in blood samples of ICU patients with a sensitivity of 75% and 73%, respectively [57]. A number of patients in this study had positive PCR results and negative simultaneous blood cultures. In the majority of these cases, microbial infection with S. aureus or E. faecalis was confirmed by microbiological isolation of these microorganisms from samples other than blood. Similar observations were made in the evaluation of an eubacterial PCR assay for detection of bacteraemia in patients with sepsis, but the sensitivity of that assay was lower (approximately 60%) [58]. This emphasizes the difference between the detection of DNA from living and/or dead bacteria by PCR and that of only viable bacteria by culture. As such, the blood culture is not the gold standard for evaluation of DNA in blood for detection of bloodstream infection. A better standard would be the presence or absence of infection (invasive or localized) with the specific microorganism, as we recently showed for S. pneumoniae DNA in blood of patients with CAP [59]. Although infection can be rapidly diagnosed with eubacterial PCR, the assay does not provide information on the species involved. Subsequent identification of the species can be achieved with follow-up with panels of specific PCR assays or with sequence analysis. Sequence analysis is especially convenient if uncommon species are suspected for which no species-specific reaction is available, but it is usually more time consuming (days). As such, the use of eubacterial PCR for the diagnosis of invasive infection remains limited to patients with persistent fever of unknown origin.

Two studies have aimed to demonstrate additional value of PCR on bronchoalveolar lavage (BAL) specimens for detection of microbial origin of ventilator-associated pneumonia (VAP), because the yield of culture in these patients is frequently reduced by prior use of antibiotics and adequate specimens may be difficult to obtain. Apfalter et al. found a high incidence of S. pneumoniae (20%) DNA and several cases of Mycoplasma pneumoniae and Chlamydophila pneumoniae DNA in BAL samples from patients with VAP, while the majority of simultaneous bacterial cultures were negative [60]. Based on clinical characteristics and S. pneumoniae DNA load in the samples, they conclude that the majority of these results should be considered as clinically relevant and not the result of contamination by nasopharyngeal flora. In another study Daubin and colleagues assessed the incidence of viral pathogens as a cause of VAP by viral PCR, viral culture and immunofluorescence assays [61]. They observed an overall prevalence of 25% for respiratory viruses in tracheobronchial aspirates of intubated adult patients with (n=39) and

Figure 2. Usefulness of microbial detection methods in relation to clinical characteristics, in this example with different types of suspected pneumonia. ADT, antigen detection test; FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction.
without VAP (n=100). Despite this high prevalence of respiratory viruses in intubated patients, in none of the cases of VAP was viral infection determined to be the aetiological cause. Both studies focused on microorganisms that are usually associated with CAP. To our knowledge, multiplex screening of BAL specimens for microorganisms involved in hospital-acquired VAP has not yet been studied. In this context it is important to determine whether PCR can discriminate between infection and colonization in BAL specimens. This might be a problem as standardization of sampling and establishment of clear reference values is difficult.

**Perspectives**

Despite the increasing use of molecular diagnostics, a surprisingly limited number of studies have assessed the potential role of rapid molecular tests in diagnosis of infection in ICU patients. The main advantages of these techniques (rapidity, unaffected by antibiotics, ability to test samples with high diversity of microbes) mean that these tests can be very useful if sepsis, invasive infection or respiratory tract infection is suspected in critically ill patients. The clinical relevance of the various molecular tests depends not only on technical features but also on patient characteristics (Table 1 & Figure 2). While culture offers broad screening for microbial pathogens, ADT, FISH and PCR assays focus on the specific detection of one or a few pathogens. As such, the choice to perform a certain molecular test is always related to the clinical picture. In addition, it is important that the result of the assay of choice influences clinical management, for example the adjustment or continuation of antifungal therapy after discrimination between *C. albicans* and non-albicans *Candida* species mentioned earlier [43,44]. In addition to an usually high clinical specificity, it is important that molecular tests have a high positive predictive value. Most of the therapeutic effects will result from identification of a specific microorganism as the cause of a patient’s illness and not from ruling out a certain microbe. To enhance usefulness in routine clinical practice, technical developments should therefore focus on the improvement of clinical sensitivity of molecular assays such as ADT and PCR for detection of DNA in blood. Most molecular tests do not yet provide data on antimicrobial susceptibility, their potential clinical impact is defined by likelihood of change of antibiotics on the basis of epidemiological knowledge of antimicrobial susceptibility of the identified pathogen, and by confirmation or exclusion of infection which may withhold or initiate further investigations into a cause of non-infectious origin. In these situations, molecular tests have a clear advantage over conventional techniques, may improve prognosis, reduce use of antimicrobials and decrease length of stay and hospital costs, and will be cost-effective [62,63]. In general, the organization of the microbiological laboratory, the number of hospital beds and the total number of tests performed are important determinants of cost-effectiveness and related to implementation of these tests in routine practice. The cost of laboratory materials and the hands-on time of technicians are reduced when multiple samples are tested simultaneously. Furthermore, technicians’ skills will improve if tests are performed on a regular basis which also reduces hands-on time. On the other hand, tests should be performed shortly after samples are obtained to have clinical impact. As such, routine implementation of rapid diagnostic tests, which are usually more expensive than traditional culture and serology, has always to be balanced with the clinical benefits of a rapid diagnosis. Few studies have been done to assess this and more studies are warranted.

One of the key developments over the past decade has been quantification of the microbial load: the amount of DNA or RNA in samples. The most well-known example is probably the HIV RNA load, but an ever-increasing number of studies report relevance of various bacterial, viral or fungal microbial loads, such as *S. aureus*, CMV, EBV and *Candida* spp. DNA load [57,64,65]. Determination of the microbial load enables monitoring of infection over time and may help to differentiate between infection, colonization and contamination [50,57,64,66]. Also, it might provide a measure of severity of infection that has prognostic impact [67,68]. Although little is known yet about the kinetics and natural course of microbial DNA in blood during infection and treatment, we expect that the microbial load will become increasingly important as a guide for the clinical management of infection in the near future.

Long-term developments focus on rapid, simultaneous identification and determination of antimicrobial susceptibility of microorganisms, i.e. with the microarray technique. With the DNA microarray technique many different oligonucleotides specific to selected genes are each spotted on a small glass slide (chip). The target DNA is labelled with a fluorescent dye as a reporter molecule. After specific hybridization of target DNA with a probe, fluorescence is measured for complementary spots. The analysis of a sample on a microarray chip generates a fluorescent specific ‘barcode’ with extensive genomic information on aspects such as housekeeping proteins, virulence factors and antimicrobial resistance determinants, in, for example, pathogens causing bloodstream infection [69]. Although the full genetic characterization of microbes with microarray could provide important clinical and research information, future developments will show whether this technique can attain a place in cost-effective routine microbiological diagnostics. The continuous unravelling of the microbial genome will eventually lead to point-of-care molecular diagnosis of infection. Interpretation of the growing burden of molecular microbial information requires specialist knowledge that should always be related to the clinical context. Therefore, good cooperation between critical care physicians, medical microbiologists, and molecular biologists becomes more and more essential to ensure optimal treatment of patients suspected of infection.
References


