Issues in Blood Culture: Contamination, and Rapid Detection of Pathogens

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Microorganisms encountered in blood

- Microorganisms present in blood, whether continuously, intermittently, or transiently are a threat to every organ in the body.
- Microbial invasion of the bloodstream can have serious invasive consequences, including shock, multiple organ failure, DIC, and death.
- Pathogens of the four major groups of microbes—bacteria, fungi, viruses and parasites may be found circulating in blood during the course of any diseases.
Microorganisms encountered in blood

**Bacteria**

- Commonly isolated bacteria from blood are:
  - gram positive cocci, including coagulase-negative staphylococci (CoNS), *S. aureus*, and *Enterococcus* species
  - and other organisms likely to be inhabitants of the hospital environment, that colonize the skin, the oropharyngeal area, and gastrointestinal of patients.
Fungi

• **Fungaemia** usually occur in immunosuppressed patients and those with serious or terminal illness.
• Fungi may gain entry to the circulatory system:
  - through damaged skin
  - from primary sites of infection or by means of intravascular catheters
  - via loss of integrity of the gastrointestinal or other mucosa

• *Candida albicans* is most commonly isolated but *Malassezia furfur* can also be isolated in neonates, receiving lipid supplemented parenteral nutrition.
• Except for *Histoplasma* which multiply in the leucocytes, fungi do not invade blood cells.
Microorganisms encountered in blood

**Parasites**

- Eukaryotic parasites may be found transiently in the bloodstream, as they migrate to other tissues or organs.
- Some examples include:
  - *Toxoplasma gondii*
  - *Microfilariae*
  - *Mansonella, Loaloa, Wuchereria, or Brugia*
- Malarial parasites invade the host erythrocytes.
- Parasites in the bloodstream are usually detected through direct visualization.
Viruses

• Although many viruses do circulate in the peripheral blood, the primary pathology relates to the infection of the target organ or cells.

• Viruses that infect blood cells include:
  – EBV
  – CMV
  – HIV

• The cell may be destroyed or damaged by viral replication, and immunologic responses of the host may also contribute to the pathogenesis.
**Interpretation of blood culture results**

**Probable pathogen**
- Growth of the same organism in repeated cultures obtained either at different times or different anatomic sites.
- Growth of certain organisms in cultures obtained from patients suspected of endocarditis such as Enterococci, or gram-negative rods in patients with clinical gram-negative sepsis.
- Growth of certain organisms (especially intestinal or urinary) and such as members of the *Enterobacteriaceae*, SPN, gram negative anaerobes, and *S. pyogenes*.
- Isolation of commensal microbial flora from blood culture obtained from patients suspected to be bacteremic (immunosuppressed)
Interpretation of blood culture results

**Probable contaminant**

- Growth of *Bacillus species, Corynebacterium species, Propionibacterium acnes*, or CNS in only one of several cultures.
- Growth of multiple organisms from only one of several cultures.
- Clinical presentation is not consistent with sepsis.
- Organism causing the infection from the primary site is not the same as that isolated from the blood culture.
Blood Culture Contamination

Overview

- A positive bloodculture can suggest a definitive diagnosis, enable the targeting of therapy against the specific organism(s) in question, and provide prognostic value.
- Like any test, however, false-positive results can limit the utility of this important tool.
- Faced with a positive blood culture result, clinicians must determine whether the organism represents a clinically significant infection associated with great risk of morbidity and mortality or a false-positive result of no clinical consequence.
- Further complicating the issue in recent years is the increasing use of central venous catheters (CVC) and other indwelling vascular access devices.
- Interpretation of culture results for patients with these devices in place is particularly challenging because while these individuals are at increased risk for bacteremia, such results may also indicate culture contamination or colonization of the line.
Blood Culture Contamination

Prevalence and Significance

• While target rates for contamination have been set at 2 to 3%, actual rates seem to vary widely between institutions, from as little as 0.6% to over 6%.

• There is also some evidence to suggest that in recent decades, these rates have been on the increase, probably due to:
  – technological advances that allow the detection of smaller quantities of living microorganisms in the blood
  – the increased use of indwelling catheters for the provision of therapy
  – changes in phlebotomy practices to minimize the risk of needlestick injuries.
Blood Culture Contamination

Financial Impact

- The financial impact of blood culture contamination has been described in a number of studies.
- Bates et al. (JAMA, 1991) found that contaminant results, compared with true-negative results, were independently associated with increased subsequent laboratory charges (20% increase) and intravenous antibiotic charges (39% increase).
- In a subsequent prospective study focused on blood culture contamination caused by coagulase-negative staphylococci, Souvenir et al. (J Clin Microbiol, 1998) reported that almost half of the patients with a false-positive result were treated with antibiotics, often with vancomycin (125). According to their estimates, the additional costs associated with this unnecessary treatment were approximately $1,000 per patient.
- Other investigators focused on the consequences of contaminated cultures in pediatric populations, finding similar results seen in adult populations.
Given these excessive costs, how can we address the problem of contaminated blood cultures?

(Hall et al, Clin Microbiol Rev 2003)
Detection of Contaminated Blood Cultures

Identity of Organism

The most important indicator of contamination when interpreting blood culture results is the identity of the organism:

<table>
<thead>
<tr>
<th>Organism(s)</th>
<th>1,585 blood culture-positive episodes from 3 U.S. hospitals</th>
<th>497,134 blood cultures from 640 U.S. institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>44.3% 82%</td>
<td>Not reported 62–63%</td>
</tr>
<tr>
<td>Corynebacterium spp. (other than C. jeikium)</td>
<td>33.4% 96%</td>
<td>Not reported 68–78%</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>0.8% 91.7%</td>
<td>Not reported 68–70% (other than B. anthracis)</td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>3.0% 100%</td>
<td>Not reported 84–85%</td>
</tr>
<tr>
<td>Viridans group streptococci</td>
<td>4.5% 49.3%</td>
<td>Not reported 32–33%</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>0.8% 76.9%</td>
<td>Not reported</td>
</tr>
</tbody>
</table>


However, it is crucial to recognize that each of these organisms can also represent true bacteremias with devastating consequences, particularly if untreated due to misinterpretation as contaminants.
Detection of Contaminated Blood Cultures
Identity of Organism (CoNS)

• In the past, coagulase-negative staphylococci were usually believed to represent contamination when isolated from blood cultures.
• In fact, **coagulase-negative staphylococci** are the most common blood culture contaminants, typically **representing 70% to 80% of all contaminated blood cultures**.
• Recently, however, studies have shown that these organisms are an increasing source of **true bacteremia in patients with prosthetic devices and central venous catheters**, although the majority of isolates of coagulase-negative staphylococci from blood cultures continue to be contaminants.
• Weinstein et al. (Clin Infect Dis, 1997) found that even though only 12.4% of coagulase-negative staphylococcal isolates were clinically significant, they ranked as the third most common cause of bacteremia because of their high prevalence.
Detection of Contaminated Blood Cultures

Identity of Organism – Polymicrobial infections

- Often, bloodstream infections involve only a single organism, prompting clinicians to sometimes conclude that a blood culture bottle that grows multiple organisms is contaminated.
- However, studies have found that 6% to 21% of all true bacteremias are polymicrobial, usually in patients in high-risk groups.
- Furthermore, multiple coagulase-negative species have been found to cause polyclonal coagulase-negative staphylococcal infections.
- Therefore, one cannot conclude that the mere presence of multiple organisms in a blood culture bottle always indicates contamination.
Detection of Contaminated Blood Cultures

Number of Positive Blood Culture Sets

• Most of laboratories cited the proportion of positive blood cultures in a series of cultures as important for interpreting blood culture results:
  – if only one set of at least two sets grows an organism known to often cause contamination, this often represents a contaminant
  – for true bacteremias, multiple blood culture sets will usually grow the same organism
  – the presence of one positive set over several cultures drawn over a period of time may also indicate contamination, although it may conversely indicate transient bacteremia

To differentiate transient bacteremia from contamination, it has been recommended that at least two sets of cultures be obtained at the same time.

• However, obtaining at least two sets is not always done, especially in pediatric patients.
• In 289.572 blood culture sets from 909 hospitals, median proportions of solitary blood cultures was of 10.1% and 12.1% among adult inpatients, 25.4% and 33.3% among adult outpatients, and 89.0% and 100% among pediatric/infant patients in the two studies, respectively (Shifman et al, Arch Pathol Lab Med, 1996).
Detection of Contaminated Blood Cultures

Time to Growth (Time to Positivity)

Another factor in determining contamination is the amount of time required for the organism to grow in the culture medium.

- Conceptually, it is thought that perhaps the blood from a bacteremic patient will have a much higher inoculum of bacteria than a contaminated culture.
- Theoretically, it follows that a larger inoculum will grow faster than a smaller inoculum, a theory that seems to have been verified in prior studies of catheter-related bloodstream infections.
- In support of this theory, several studies have shown that cultures that become positive more than 3 to 5 days after incubation have been more likely to represent contaminants.
- A time to positivity of $\leq 15$ h had a positive predictive value of 84% for true infection in children (Haimi-Cohen et al, Paediatr Infect Dis 2003).

However, existence of discordant results suggest that this technological variable should not be relied upon to distinguish contaminants from pathogens in blood cultures (Weinstein, J Clin Microbiol 2003).
Given these excessive costs, how can we address the problem of contaminated blood cultures?

(Hall et al, Clin Microbiol Rev 2003)

<table>
<thead>
<tr>
<th>Approach</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detecting contaminants</td>
<td>Given that contamination can likely never be eliminated, having reliable factors to help identify true positives vs false positives is critical for patient management and population-based surveillance.</td>
</tr>
<tr>
<td>Prevention</td>
<td>Reducing contamination rates will improve the specificity of the blood culture and result in a higher PPV, resulting in a significantly more useful test.</td>
</tr>
<tr>
<td>Supporting optimal use of blood cultures</td>
<td>Reducing the use of blood cultures in patients with a very low likelihood of bacteremia will result in a higher PPV and reduced costs associated with contamination; pretest probability of bacteremia can be estimated using population-based studies of disease prevalence or clinical prediction rules.</td>
</tr>
</tbody>
</table>
Preventing Contamination

Skin Preparation

• The most common source of contaminated percutaneous blood cultures is often thought to be the skin of the patient at the site where the cultures are obtained. However, not all CoNS are from skin’s patient ...

• One study (Viagappan et al, J Hosp Infect 1995) attempted to find the source of CoNS contaminants by performing molecular analysis to compare each blood isolate to isolates obtained from swabs of the patient and from the individual who obtained the culture.
  – Of 19 patients with contaminated cultures, 6 of the isolates were able to be matched to swabs, and all 6 matching swabs came from the patient, implying that the most common source of contamination is the patient’s own skin flora.
  – However, it is important that no matching sources were found for 13 of the 19 isolates.

• Inadequate skin preparation is thought to be the most common cause of blood culture contamination

• However, skin antisepsis cannot entirely prevent the contamination of blood cultures from skin flora because as many as 20% of skin-associated bacteria have been found to survive disinfection, as found by culturing skin samples harvested with a sterile surgical technique.
Preventing Contamination

Skin Preparation

• Several barriers can prevent the skin antisepsis technique from being effective for preventing contamination:
  – poor penetration of disinfectant
  – presence of bacteria in deeper skin layers
  – time required for the antiseptic to have maximal effect (i.e., PVP-I preparations require 1.5 to 2 min of contact time to have a maximal antiseptic effect, whereas tincture of iodine requires 30 sec)

• Many studies have been performed to determine the best skin antiseptic product to use for blood culturing. Perhaps the most commonly studied and traditionally used skin antiseptic for blood culturing is povidone-iodine, an iodophor.

• Although controversial, some experts recommend that the culture site be firstly prepared with 70% isopropyl or ethyl alcohol and allowed to air dry, then a second prep should be performed using 1 to 2% tincture of iodine or 10% povidone-iodine (Magadia et al, Infect Dis Clin N Am 2001).
Preventing Contamination

Culture Bottle Preparation

- Although data are limited, it is standard practice to disinfect the tops of the culture bottles before inoculating them with blood, since the rubber stopper on each bottle is not sterile.
- In the CAP Q-Probes study of 640 institutions, 95.5% of organizations routinely applied an antiseptic to the top of the culture bottle before inoculating the bottle (113), achieving significantly lower contamination rates (2.3%) than those that did not prep the bottle tops (3.4%).
- It has been recommended that individuals **should not use iodine alone** because it may cause erosion of the rubber stopper during incubation, thereby introducing contaminants.
- Some institutions use **alcohol**, whereas others use an **iodine solution** that is **allowed to dry and is then wiped off with fresh alcohol** prep prior to inoculating the bottle.
Preventing Contamination

Obtaining Cultures Percutaneously instead of via Vascular Catheters

• As stated above, cultures obtained from vascular catheters can be quite difficult to interpret.

• However, this technique remains a very common practice for many reasons:
  – to prevent inflicting pain on patients by drawing cultures from a catheter instead of percutaneously
  – to decrease the likelihood of inducing transient bacteremia by phlebotomy, especially for highly immunosuppressed patients
  – for the neonatal population, the difficulty of venous access is a real problem
  – fear to cause nosocomial volume depletion or anemia by drawing too many blood cultures, a potential issue with neonates or profoundly anemic patients

• Despite these good intentions, there are many undesirable consequences of this practice:
  – the requirement for more cultures for clarification
  – more diagnostic studies
  – the unnecessary use of antibiotics with the associated potential for allergic reactions
  – unanticipated drug interactions, or adverse drug events
  – if falsely positive culture results from vascular catheters are misinterpreted, unnecessary and prolonged vascular access for intravenous antibiotics may be a consequence
Given these excessive costs, how can we address the problem of contaminated blood cultures?

(Hall et al, Clin Microbiol Rev 2003)
Supporting Optimal Use of Blood Cultures

• Blood cultures are frequently ordered tests. Data from literature suggested that over 20% of hospitalizations involve at least one blood culture.

• The high utilization of blood culture testing can be partially attributed to at least three important reasons:
  – the difficulty that most clinicians have in predicting the risk of bacteremia
  – their low threshold for ordering the test, given the significant risk of morbidity and mortality associated with this condition
  – additionally, for some specific clinical conditions, such as patients admitted to the hospital with community-acquired pneumonia, professional or governmental organizations have recommended universal blood culture testing (Mandell, Clin Infect Dis 2003).

• The pretest probability for bacteremia greatly influences the positive predictive value of a blood culture result. Thus, several clinical prediction rules for bacteremia have been planned in a wide variety of patient populations in an effort to assist decision-making regarding diagnostic workup and management.

• The scientific community has also assessed the rates of bacteremia in a wide variety of patient populations to better understand disease prevalence, potentially allowing the development and refinement of consensus guidelines for specific clinical scenarios.
Rapid techniques to detect Bloodstream Infections
Bloodstream Infections

- Bacteremia and Candidemia are Serious Infections
  - >50% of bloodstream infections are hospital-associated
  - Patient costs: $28,000 (community) – $105,000 (nosocomial)
  - Mortality: 14% (community) – 34% (nosocomial)
- Patients treated empirically, e.g. vancomycin to cover for Gram+
- Early and effective antibiotic therapy is crucial for patient outcome
- Traditional diagnostics (Dx) can take 48-72 hours

Diekema et al., J Clin Microbiol. 2003
Bloodstream Infections – Rapid Dx

“If you were septic, would you wait 48-72 hours to know if you are receiving appropriate or unnecessary antibiotic therapy?”

Dr. Graeme Forrest. Infectious Diseases. University of Maryland Medical Center. ICAAC 2006, San Francisco, CA
Key Issue

In most hospitals today, by the antimicrobial susceptibility test results from blood culture are available to the physician, the results are more likely to answer the question

– “Did the physician choose the correct empiric therapy?”

rather than

– “Which antimicrobial agent is best for treating this patient’s infection?”
The primary mission of a clinical bacteriology laboratory is to assist clinicians in the diagnosis and treatment of infectious diseases, and to support infection control specialists in their tasks. In many instances, however, the delay between the collection of the specimen and the results of the bacterial culture makes the latter unhelpful for the clinician.

There is a ‘need for speed’, as rapid identification of a pathogen is of prime importance for effective patient management. It gives a clue towards the primary site of infection, and often reduces the use of empirical broad-spectrum antibiotic therapy to a more narrow pre-emptive treatment, based on the usual susceptibilities (or natural resistance) of the pathogen.
Initially, most infections are treated empirically until the causative agents and their susceptibility profile are known. As soon as results of determination and susceptibility tests become available, the antibiotic regimen can be streamlined. Since administration of appropriate antimicrobial agents is correlated with a decrease in mortality, shortening the period in which empirical therapy is given may result in a better outcome for the patient.

Determination and susceptibility testing of microorganisms usually takes 24–48 h after initial growth in a routine laboratory setting. According to a study by Kerremans et al (JAC, 2008), the average time it takes for a physician to receive susceptibility testing results is 40 hours.

With the newest diagnostic methods, however, identification and susceptibility testing can now be performed within one working day. It is to be expected that modification of antibiotic therapy to narrow spectrum antibiotics or to adequate antibiotics can be made earlier using these rapid techniques, and that these techniques will contribute to improved patient management.

Rapid bacterial identification and susceptibility tests can lead to earlier microbiological diagnosis and pathogen-directed, appropriate therapy. Hospitalized individuals, as well as those in the community, may benefit from the prudent use of antibiotics.
Initial inappropriate therapy in patients with serious infections is associated with higher mortality.

**Bar Chart:**
- **Rello et al.**
  - Infection-related mortality
- **Kollef et al.**
  - Crude mortality
- **Ibrahim et al.**
  - Infection-related mortality
- **Luna et al.**
  - Crude mortality

**Mortality (%)**

**References:**
The Importance of Optimizing Therapy Early

- Kollef et al. performed a prospective study of 895 hospitalized, febrile, adult patients and compared 30-day, all-cause mortality rate and mean length of hospital stay for patients who received inappropriate (n=319) or appropriate (n=576) initial antimicrobial treatment.

- Results: Significantly higher mortality rates (20.1% vs. 11.8%; P=.001) and longer lengths of hospitalization (at least 2 days longer; P=.002) were observed for patients receiving inappropriate initial therapy.

In a retrospective cohort study of records for 2,154 patients with septic shock, increasing delays in the initiation of effective therapy after the onset of persistent or recurrent hypotension were associated with a significantly ($P < .0001$) increased risk of death (figure). Further, median time to initiation of effective therapy was a strong predictor of mortality. In fact, the survival rate with therapy initiated within the first hour was 79.9%; each additional hour that therapy was delayed resulted in a mean decrease in survival rate of 7.6%. The survival rate with therapy initiated in the sixth hour was 42%.

Time to Culture and Susceptibility Test Results

- Final culture and AST report to doctor
- Susceptibility test (fastidious)
- Susceptibility test
- Prelim report
- Culture

Blood vial positive

Hours: 24, 36, 48, 72, 96 (4 days!!!)
Fig. 94

Esam. cultura: schema operativo per la ricerca dei microrganismi.

Legenda: 1) a: limite da raggiungere con il campione di sangue; b: limite da raggiungere con il terreno liquido
2) T.B.I. = Test biochimici di identificazione  3) MSA = Mannitol salt agar
Identification of Organisms in Positive Blood Cultures

- Most laboratories undertake some sort of rapid testing to identify organisms in positive blood cultures
  - Gram stain (acridine orange, calcifluor white) results included in preliminary report
  - Tube coagulase test for staphylococci
  - Commercial or in house meca PCR (for MRSA)
  - Direct inoculation of commercial ID/AST panels
  - Information called to physician, if possible

- Impact of rapid testing is limited mostly to staphylococci
Sepsis - Blood specimens

- Direct PCR
- PCR/ESI-Mass Spec
- Film array
- Microarray
- + Blood Culture Bottle
- Gram stain
- Direct AST
  - Automated AST
  - Disk Diffusion
- Conventional Culture and AST
- PCR
- PNA-FISH
- MALDI-TOF
Roche SeptiFast™ Test

- Highly specific – able to detect <100 organisms per ml blood
- Detects microorganisms that cause >90% of all bloodstream infections
- Detecting for Internal Transgenic Spacer (ITS, ITS1, ITS2), a piece of non-functional RNA situated between structural ribosomal RNAs on a common precursor transcript
- Real Time PCR on Light Cycler Platform
- 3 parallel reactions: gram-positive, gram-negative, and fungus
- Melting curve analysis of end product ensures proper sequence
- Rapid, automated results in less than 5 hours

<table>
<thead>
<tr>
<th>Gram (-)</th>
<th>Gram (+)</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td><em>Staphylococcus aureus</em></td>
<td><em>Candida albicans</em></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae / oxytoca</em></td>
<td>*CoNS (Coagulase negative</td>
<td><em>Candida tropicalis</em></td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td><em>Staphylococci</em>)</td>
<td><em>Candida parapsilosis</em></td>
</tr>
<tr>
<td><em>Enterobacter cloacae / aerogenes</em></td>
<td><em>Streptococcus pneumoniae</em></td>
<td><em>Candida krusei</em></td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td><em>Streptococcus spp.</em></td>
<td><em>Candida glabrata</em></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td><em>Enterococcus faecium</em></td>
<td><em>Aspergillus fumigatus</em></td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td><em>Enterococcus faecalis</em></td>
<td></td>
</tr>
<tr>
<td><em>Stenotrophomonas maltophilia</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Sample → Lysis → Extraction → PCR → Report

less than 6 h
The LightCycler® SeptiFast Test Workflow

1. Whole Blood 1.5 ml
2. 1 ml
3. 300 µl Eluate
4. 50 µl / Test
5. The LightCycler® SeptiFast Kit
6. The LightCycler® 2.0 Instrument SW4.05
7. Pathogen Identification
8. Gram(+) / Gram(-) / Fungi
9. SeptiFast Lys Kit
10. SeptiFast Prep Kit
11. SeptiFast Identification Software (SIS)
# Diagnosing blood stream infections

**Earlier with SeptiFast (REAL TIME PCR)**

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoculture</td>
<td>Gram</td>
<td>Species</td>
<td>Resistance</td>
</tr>
<tr>
<td><strong>6 h</strong></td>
<td>SeptiFast</td>
<td>Species</td>
<td>Optionally: mecA Resistance (+2.5 h)</td>
</tr>
</tbody>
</table>

## Note:

*PCR is not intended to replace culture*
Molecular Methods for Bacteremia: SeptiFast- Direct detection in Blood

- Direct detection of 25 bacterial and fungal species in blood. Three studies gave conflicting results but all agree this cannot replace traditional blood cultures. Processing time ~6 hrs.
- Westh et al. (2009) showed reference method detected 96 organisms while SeptiFast detected 186. For SeptiFast only, 57 organisms not recovered from other body site.
- Von Lilienfeld-Toal et al. (2009) showed 71% agreement between blood culture and SeptiFast, but for 5 of 6 Aspergillus + samples, gallactomannan also positive
- Tsalik et al (2010) reported SeptiFast positive less often than culture. SeptiFast failed to detect pneumococci.

Multiplex real-time PCR and blood culture for identification of bloodstream pathogens in patients with suspected sepsis

H. Westh\textsuperscript{1,2}, G. Lisby\textsuperscript{1,2}, F. Breyssse\textsuperscript{1}, B. Böddinghaus\textsuperscript{1}, M. Chornarat\textsuperscript{2}, V. Gant\textsuperscript{2}, A. Goglio\textsuperscript{1}, A. Raglio\textsuperscript{2}, H. Schuster\textsuperscript{1}, F. St H. Wissing\textsuperscript{2} and A. Hoefft\textsuperscript{2}

### TABLE 1. Multiplex PCR test SeptiFast master list; the bacteria and fungi listed can be detected by a three-capillary multiplex real-time LightCycler 2.0 system (limits of detection of microorganisms are described in the footnotes\textsuperscript{a})

<table>
<thead>
<tr>
<th>Gram-negative</th>
<th>Gram-positive</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli\textsuperscript{d}</td>
<td>Staphylococcus aureus\textsuperscript{c}</td>
<td>Candida albicans\textsuperscript{b}</td>
</tr>
<tr>
<td>Klebsiella (pneumoniae/oxacola)\textsuperscript{a}</td>
<td>Coagulase-negative staphylococci\textsuperscript{c}</td>
<td>Candida tropicalis\textsuperscript{b}</td>
</tr>
<tr>
<td>Serratia marcescens\textsuperscript{a}</td>
<td>Streptococcus pneumoniae\textsuperscript{c}</td>
<td>Candida parapsilosis\textsuperscript{b}</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>Streptococcus spp.\textsuperscript{a}</td>
<td>Candida glabrata</td>
</tr>
<tr>
<td>(Acinetobacter/aeurogenes)\textsuperscript{b}</td>
<td>Proteus mirabilis\textsuperscript{b}</td>
<td>Enterococcus faecium\textsuperscript{a}</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa\textsuperscript{b}</td>
<td>Enterococcus faecalis\textsuperscript{a}</td>
<td>Aspergillus fumigatus\textsuperscript{b}</td>
</tr>
<tr>
<td>Acinetobacter baumannii\textsuperscript{c}</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia\textsuperscript{b}</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Limit of detection of microorganisms as described in the package insert of the commercial assay; all microorganisms in the SeptiFast master list found at concentrations of 100 CFU/mL.

\textsuperscript{b} Microorganisms found in 2020 analysis at 3 CFU/mL.

\textsuperscript{c} Microorganisms found in 2020 analysis at 30 CFU/mL.

### TABLE 2. Number of microorganisms/isolates detected with SeptiFast or blood culture

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Any system</th>
<th>Both systems</th>
<th>SeptiFast only</th>
<th>Blood culture only</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>32</td>
<td>12</td>
<td>20</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>16</td>
<td>5</td>
<td>9</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>14</td>
<td>5</td>
<td>7</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>14</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>11</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>NS</td>
</tr>
</tbody>
</table>

### TABLE 3. Isolat culture and SeptiFast

<table>
<thead>
<tr>
<th>SeptiFast</th>
<th>Positive</th>
<th>Negative</th>
<th>Contaminant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>27</td>
<td>10</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>12</td>
<td>2</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella pneumoniae/oxytoca</td>
<td>11</td>
<td>0</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>17</td>
<td>2</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>12</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Not in SeptiFast master list</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Subtotal</td>
<td>198</td>
<td>50</td>
<td>124</td>
<td>24</td>
</tr>
</tbody>
</table>

### Table 4. Significantly more microorganisms detected by SeptiFast culture and SeptiFast.

<table>
<thead>
<tr>
<th>Contaminant microorganisms/isolates</th>
<th>SeptiFast</th>
<th>Blood culture only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus spp.</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Propionibacterium spp.</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>1</td>
</tr>
</tbody>
</table>

NS, not significant.

\textsuperscript{a} The McNemar test was used for testing the difference between paired proportions (SeptiFast only vs. blood culture only).

\textsuperscript{b} Not included in the SeptiFast master list.
The first description of the use of MS for bacterial identification was in 1975. Mass spectral analysis of lipids such as menaquinones in the 1980s witnessed the restructuring of many genera.

However, it took a long time for MS technology to enter routine microbiology, because of the limited mass range (<1500 Da), which restricted its applications to small molecules, such as various bacterial lipids.

Proteins are orders of magnitude greater, and their analysis by MS had to await the arrival of soft ionization techniques such as MALDI and electrospray ionization (ESI).

By the mid-1990s MALDI-TOF MS was being used for the identification of bacteria in research settings.

A few years later, the first complete database for bacterial identification, based on the analysis of surface molecules of bacterial cells, was reported. This approach was expected to allow not only the identification of bacteria to the species level, but also the identification of key surface-associated molecules, such as virulence factors.

However, it was not well suited for routine bacteriology, as it necessitated rigorous standardization, owing to the variation in surface proteins.

A more robust approach for routine species identification was then developed, using MALDI-TOF MS but involving the use of a different matrix.

The change of matrix permitted the ionization of mainly ribosomal proteins, which are more conserved than surface proteins and are not significantly affected by culture conditions.
Matrix-assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS)

**PRINCIPLE**

- A thin layer of bacterial cells is spread across the well of a conductive metallic plate (the ‘target’).
- Each specimen is then covered with a solution of acidic molecules (the ‘matrix’), which creates a mixture with the analyte molecules.
- The ‘target’ is then placed in the instrument, and brief laser pulses hit the mixture. The small desorbed and ionized molecules are accelerated through an electrostatic field and drift through a field-free tunnel until they reach the mass spectrometer’s detector.
- Molecules of different masses and charges will ‘fly’ at different speeds (hence the term ‘time-of-flight’).
- The result is a spectral signature that will be searched for in the appropriate database for the identification of the organism to the genus or species level.
Application and use of various mass spectrometry methods in clinical microbiology

S. Emonet¹, H. N. Shah², A. Cherkaoui¹ and J. Schrenzel¹

FIG. 1. Time to identification or typing: the role of mass spectrometry. MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MALDI-RE, matrix-assisted laser desorption ionization–resequencing; PCR-ESI-MS, PCR–electrospray ionization mass spectrometry; ID, identification; MLST, multilocus sequence typing; AB, antibiogram.
Matrix-assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS)

MICROBIAL IDENTIFICATION

- This new (molecular instead of phenotypic) method of identification of microorganisms, has proven to be reliable and safe for the routine diagnostic identification of Enterobacteriaceae, a majority of non-fermenters, staphylococci, enterococci, b-haemolytic streptococci, and anaerobes (especially Actinomyces spp.).

- Some bacteria and most yeasts are more difficult to identify with MALDI-TOF MS, mainly for the quality of the spectrum obtained, but also for the quality of the database or the limits of a technique based mainly on ribosomal proteins (e.g. the difficulty in distinguishing S. pneumoniae from S. mitis with MALDI-TOF MS is congruent with 16S rDNA sequencing).

MALDI-TOF MS on positive blood culture vials

- MALDI-TOF MS reduces the time needed for identification of a positive blood culture vial, especially in the case of rapidly growing organisms (Gram-positives or Enterobacteriaceae).

- It simply requires the immediate seeding of blood sheep agar medium, followed by a 2–3-h incubation step, in order to obtain a thin layer of colonies, sufficient for MS.

- Thereafter, identification is obtained in 1 min by MALDI-TOF MS. In most cases, the clinician will obtain the identification of the pathogen at least 1 day earlier.

- In the case of slow-growing pathogens (i.e. anaerobes), MALDI-TOF MS is performed directly on the positive blood for identification of a pathogen.

- In 2010, many studies have been published showing good results with MALDI-TOF MS performed directly on blood vials, by using more or less simple protocols to get rid of blood, medium and human proteins. These extraction protocols can usually be performed within 1 h.
ElectroSpray Ionization Mass Spectrometry (ESI-MS)

**PRINCIPLE**
- The sample (usually DNA amplicons) is dissolved in a solvent and injected in a conductive capillary, where high voltage (V) is applied, resulting in the emission of aerosols of charged droplets of the sample.
- The latter are sprayed through compartments with diminishing pressure (P), resulting in the formation of gas-phase multiple-charged analyte ions, which then are detected by the spectrometer.
- ESI-MS allows high resolution, up to base composition analysis of amplicons.
Application and use of various mass spectrometry methods in clinical microbiology

S. Emonet¹, H. N. Shah², A. Cherkaoui¹ and J. Schrenzel¹

**FIG. 1.** Time to identification or typing: the role of mass spectrometry. MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MALDI-RE, matrix-assisted laser desorption ionization—resequencing; PCR-ESI-MS, PCR—electrospray ionization mass spectrometry; ID, identification; MLST, multilocus sequence typing; AB, antibiogram.
PCR-ESI-MS (PLEX-ID, Abbott Molecular)

MICROBIAL IDENTIFICATION AND TYPING

- The identification mode of the PCR-ESI-MS tool permits rapid and semiquantitative identification directly from a specimen, with a result being provided within 4–6 h.
- Because of the relatively high multiplexing potential of the method, it could provide:
  1. Support for infection control specialists (e.g. partial typing, for characterization and tracking of pathogens causing an epidemic).
  2. Detection of virulence or resistance (mecA gene for methicillin resistance, the vanA and vanB genes for vancomycin resistance in enterococci, and the blaKPC gene for resistance to carbapenems).
  3. A tool for analysis for syndromic panels of infection, such as respiratory viruses (i.e. PLEX-ID for the pandemic H1N1 virus in 2009).
- Clinical researchers may find wider applications for this technology, similar to microarrays but faster. It has even been proposed that PLEX-ID may allow precise genotyping with the same primers as used for MLST.
<table>
<thead>
<tr>
<th>Applications</th>
<th>MALDI-TOF MS (Brooker and Shimizu)</th>
<th>MALDI-RE SEQUENOM (iSeq)</th>
<th>PCR-ESI-MS (PLEX-ID, Abbott)</th>
<th>Conventional capillary sequencing</th>
<th>Second-generation sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacterial ID</td>
<td>Virulence and resistance</td>
<td>Typing</td>
<td>Bacterial ID</td>
<td>Typing</td>
</tr>
<tr>
<td>Analytical sensitivity</td>
<td>Requires culture, but new research protocols permit 1000 [34]</td>
<td>Using special protocols, provided the gene is expressed [35,36]</td>
<td>Excellent, based on the PCR conditions [17]</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Good</td>
<td>To be determined in clinical studies, but considered to be high (10–100)</td>
<td>High</td>
<td>Moderate when relying on broad-range primers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Excellent, Clinical specificity to be evaluated</td>
<td>High</td>
<td>Excellent, reference method for bacterial taxonomy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Moderate</td>
<td>High</td>
<td>Highest</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Currently the fastest method (4–6 h after sampling lysing bacterial cultures)</td>
<td>High</td>
<td>Highest</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Moderate, especially if compared with phenotypic tests</td>
<td>High</td>
<td>Highest</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Highest</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Highest</td>
</tr>
<tr>
<td>Assay time to result</td>
<td>Minimal 1–2 min per sample, starting from a colony or even directly from urine [32]</td>
<td>High 1–2 min per sample, starting from a colony</td>
<td>Rapid as compared with MLST</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Highest</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Highest</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Highest</td>
</tr>
<tr>
<td>Multiplexing</td>
<td>Not possible</td>
<td>Possible</td>
<td>Possible even with a high throughput</td>
<td>Possible</td>
<td>Possible</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Possible</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Possible</td>
</tr>
<tr>
<td>Viruses</td>
<td>Not possible</td>
<td>Not possible</td>
<td>Possible [9]</td>
<td>Possible</td>
<td>Possible</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Possible</td>
</tr>
<tr>
<td>Detection of unknown pathogen</td>
<td>Could be pictured in a dendrogram, but lack of specificity. Needs an isolated colony</td>
<td>Not possible</td>
<td>Can be precisely mapped on a taxonomy tree. Moreover, genome mapping allows epidemiological studies</td>
<td>Possible</td>
<td>Possible</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Possible</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Possible</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Possible</td>
</tr>
<tr>
<td>Running costs</td>
<td>Minimal</td>
<td>Minimal</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High</td>
</tr>
</tbody>
</table>

ID, identification; MALDI-RE, matrix-assisted laser desorption ionization–resequencing; MALDI-TOF MS, matrix-assisted laser desorption ionization–time-of-flight mass spectrometry; MLST, multilocus sequence typing; NA, not applicable; PCR-ESI-MS, PCR–electrospray ionization mass spectrometry.
Detection and Identification of *Ehrlichia* Species in Blood by Use of PCR and Electrospray Ionization Mass Spectrometry

Mark W. Eshoo,¹ Chris D. Crowder,¹ Haijing Li,² Heather E. Matthews,¹ Shufang Meng,² Susan E. Sefers,² Rangarajan Sampath,¹ Charles W. Stratton,²,³ Lawrence B. Blyn,¹ David J. Ecker,¹ and Yi-Wei Tang²,³,*

**Whole-blood specimens** from patients suspected of having a tick-borne illness were extracted using the NucliSense Easy Mag System and the DNA amplified using 16 primer pairs. The primers included 4 broad-range primer pairs targeting the 16S and 23S DNA. The other primers were selected based on their coverage of groups of known tick-borne pathogens. Products were analyzed by PCR electrospray ionization mass spectrometry system.
Examples of Current Diagnostic Tests Used on Blood Cultures for Staphylococci

- **Gram**: Positive Cocci in Clusters
- **PNA-FISH**: ~1.5 h, Green fluorescence = *Staphylococcus aureus* (High complexity)
- **Commercial PCR**: (In microbiology lab)
  - MRSA/SA (2-3 hrs batch) (High complexity)
  - Microbiology or Core lab
  - MRSA/SA (1-2 h on demand) (Moderate complexity)
Xpert System - Cepheid

**TURN-AROUND TIMES OF POSITIVE RESULTS CALLED TO ICU**

<table>
<thead>
<tr>
<th>Test</th>
<th>Number</th>
<th>Mean Turn Around Time (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpert MRSA Test – Positive Result</td>
<td>125</td>
<td>6.9</td>
</tr>
<tr>
<td>Cultures – Positive Results</td>
<td>75</td>
<td>35.8</td>
</tr>
</tbody>
</table>

*Using culture] would equate to a additional 44 HAI transmissions... which would indicate that the use of Xpert MRSA could save up to 11 MRSA infections...

**XPERT MRSA VS. REFERENCE CULTURE METHOD**

<table>
<thead>
<tr>
<th>Culture</th>
<th>Xpert MRSA</th>
<th>+</th>
<th>-</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>182</td>
<td>44</td>
<td>226</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>29</td>
<td>819</td>
<td>848</td>
<td></td>
</tr>
<tr>
<td></td>
<td>211</td>
<td>863</td>
<td>1074*</td>
<td></td>
</tr>
</tbody>
</table>

* Three specimens did not give Xpert MRSA results on 2 attempts

**POSITIVE AGREEMENT:** 86.3%
**NEGATIVE AGREEMENT:** 96.6%

PPV*: 80.5%
NPV*: 96.6%

**XPERT MRSA VS. CHROMOGENIC CULTURE MEDIA**

- Xpert MRSA: 86%
- MRSA-ID: 51%
- CHROMagar MRSA: 59%
- MRSA SELECT: 65%
Rapid Detection of *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* (MRSA) in Wound Specimens and Blood Cultures: Multicenter Preclinical Evaluation of the Cepheid Xpert MRSA/SA Skin and Soft Tissue and Blood Culture Assays


---

### TABLE 1. Performance of Xpert MRSA-*S. aureus* assay for SSTIs and for BC bottles in comparison with broth-enriched routine culture

<table>
<thead>
<tr>
<th>Source and organism</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SSTI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRSA</td>
<td>97.1 (34/35)</td>
<td>96.2 (76/79)</td>
<td>91.9 (34/37)</td>
<td>98.7 (76/77)</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>100 (55/55)</td>
<td>96.6 (57/59)</td>
<td>96.5 (55/57)</td>
<td>100 (57/57)</td>
</tr>
<tr>
<td><strong>BC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRSA</td>
<td>98.3 (57/58)</td>
<td>99.4 (346/348)</td>
<td>96.6 (57/59)</td>
<td>99.7 (346/347)</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>100 (120/120)</td>
<td>98.6 (282/286)</td>
<td>96.7 (120/124)</td>
<td>100 (282/282)</td>
</tr>
</tbody>
</table>

Results available in just over an hour
An Antimicrobial Stewardship Program’s Impact with Rapid Polymerase Chain Reaction Methicillin-Resistant Staphylococcus aureus/S. aureus Blood Culture Test in Patients with S. aureus Bacteremia

Karri A. Bauer, Jessica E. West, Juan-Miguel Balada-Llasat, Pravati Pancholi, Kurt R. Stevenson, and Debra A. Goff

Table 2. Demographic and Clinical Characteristics of Patients in the Study Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pre-rPCR period (n = 74)</th>
<th>Post-rPCR period (n = 82)</th>
<th>P^&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years ± SD</td>
<td>57 ± 16.7</td>
<td>56 ± 16.0</td>
<td>.51</td>
</tr>
<tr>
<td>Male sex</td>
<td>43 (58)</td>
<td>40 (49)</td>
<td>.26</td>
</tr>
<tr>
<td>Penicillin allergy</td>
<td>9 (12)</td>
<td>10 (12)</td>
<td>.60</td>
</tr>
<tr>
<td>Surgical service</td>
<td>24 (32)</td>
<td>10 (12)</td>
<td>.003</td>
</tr>
<tr>
<td>ICU&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49 (66)</td>
<td>55 (67)</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>MRSA infection</td>
<td>44 (59)</td>
<td>37 (45)</td>
<td>.08</td>
</tr>
<tr>
<td>ID consult</td>
<td>41 (55)</td>
<td>49 (60)</td>
<td>.63</td>
</tr>
<tr>
<td>Time to ID consult, mean days ± SD (n = 90)</td>
<td>9 ± 16.4</td>
<td>3 ± 2.4</td>
<td>.05</td>
</tr>
<tr>
<td>Hospital mortality</td>
<td>19 (26)</td>
<td>15 (19)</td>
<td>.33</td>
</tr>
<tr>
<td>Hospital costs by department</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pharmacy, mean USD ± SD</td>
<td>10,375 ± 21,221</td>
<td>7457 ± 13,250</td>
<td>.08</td>
</tr>
<tr>
<td>Microbiology laboratory, mean USD ± SD</td>
<td>6808 ± 10,290</td>
<td>5081 ± 6677</td>
<td>.13</td>
</tr>
<tr>
<td>Room and board</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICU, mean USD ± SD</td>
<td>27,667 ± 35,777</td>
<td>17,737 ± 21,464</td>
<td>.03</td>
</tr>
<tr>
<td>Non-ICU, mean USD ± SD</td>
<td>12,210 ± 13,741</td>
<td>10,117 ± 10,832</td>
<td>.32</td>
</tr>
<tr>
<td>Other, mean USD ± SD</td>
<td>25,464 ± 36,633</td>
<td>16,400 ± 20,031</td>
<td>.02</td>
</tr>
<tr>
<td>Total hospital costs, mean USD ± SD (n = 154)</td>
<td>69,737 ± 96,050</td>
<td>48,350 ± 55,196</td>
<td>.03</td>
</tr>
</tbody>
</table>

Clinical Infectious Diseases 2010; 51(9):1074–1080
Barriers to Implementation of Molecular Diagnostics

- Physician acceptance of rapid results often requires additional interventions
  - Carver et al. (JCM 2008;46:2381-3) showed that even when meCA results were available within 2 hrs of identifying a positive blood culture with GPCC, therapy only changed when a clinical pharmacist delivered the data. Time to optimal therapy reduced by 25 hrs
  - Nguyen et al. (JCM 2010;48:785-90) showed that a meCA PCR test reduced vancomycin usage (patients switched off vancomycin for MSSA bacteremia in 3 days versus 5 days) and LOS was reduced by 3 days when therapy optimized. Physician education necessary.
Additional PNA-FISH Assays for Blood Culture Analysis

- Gram-negative sepsis
- Pseudomonas aeruginosa
  Versus
  E. coli / Klebsiella (green)

- Fluconazole or not?
  Green C. albicans / C. parapsilosis (Y),
  Yellow C. tropicalis (?)
  Red C. glabrata and/or C. krusei (N)
PNA-FISH

- **Peptide Nucleic Acid Fluorescence In Situ Hybridization**

- Fluorescent PNA probe hybridizing to ribosomal RNA

- PNA molecules are DNA mimics in which the negatively charged sugar-phosphate backbone of DNA is replaced with a non-charged polyamide or “peptide” backbone. Because of the non-charged backbone, PNA probes do not encounter the electrostatic repulsion allowing them to hybridize rapidly and tightly to nucleic acid targets.

- Species identification from positive blood cultures in 2 h

- For *in vitro* diagnostic use (FDA cleared, CE-mark):
  - *S. aureus*/CNS PNA FISH (KT005)
  - *E. faecalis*/OE PNA FISH (KT003)
  - *C. albicans*/C. *glabrata* PNA FISH (KT006)
PNA-FISH

- Once a blood culture turns positive, a Gram stain is performed and based on the results the appropriate PNA FISH test is selected.

- Following the Gram stain result, a drop from the positive blood culture is fixed onto a microscope slide. PNA probe is added and hybridizes to the rRNA within the target microbes (S. aureus, C. albicans, E. faecalis, other enterococci, etc.). Excess probe is removed during a stringent wash step and the slides are visualized using fluorescence microscopy. Fluorescing cells identify the target microbe while non-florescence indicates the presence of a different microbe in the blood culture.
Traditional Dx vs PNA-FISH

Day 0
- Phlebotomy

Day 1
- Positive Blood Culture
- Gram Stain
- Call to physician

Day 2
- Traditional Species ID

Day 3
- Antibiotic Susceptibility

Day 4
- Targeted Rx

Traditional Dx
- Empiric Rx
- Broad-spectrum Rx

PNA FISH
- Empiric Rx
- Targeted Rx
PNA-FISH  AdvanDx

Positive Blood Culture

Gram Stain

Results (2.5 Hrs.)

S. aureus/CNS

E. faecalis/OE

C. albicans/C. glabrata

S. aureus

E. faecalis

C. albicans

CNS

Other enterococci

C. glabrata

Non-staphylococci

Non enterococci

Non-C. albicans

Non-C. glabrata
**PNA FISH® for Blood Cultures: Assay Procedure**

### Preparation of Smears
- **Add positive blood culture**
- **Fix cells by heat, flame or methanol fixation**
- **Prepare and pre-heat Wash Solution and dH2O**

Add one drop of **Fixation Solution** to well of the microscope slide. For GNR samples, use GN Fixation Solution.

Transfer 10µl or a small drop from a ventillation needle of culture to the Fixation Solution and mix gently to emulsify.

Options to fix smears:
- Heat for 20 min. at 55-80°C
- Allow smears to dry and fix by flame fixation
- Allow smears to dry and fix by methanol fixation

Prepare working strength Wash Solution and dH2O in separate Staining Dishes. Add cover and start pre-heating in water bath (55 ± 1°C).

### Hybridization
- **Add PNA probe**
- **Add PNA probe to PNA FISH Control Slide**

Add one drop of PNA probe to the well on the microscope slide with the fixed smear. Add coverslips. Avoid air bubbles.

Add one drop of PNA probe to each well of the PNA FISH Control Slide. Add coverslips. Avoid air bubbles.

### Hybridization (cont.)
- **Hybridize**

Incubate for 30 minutes at 55 ± 1°C.

Transfer Gram-negative rod slides to slide rack. Immerse slide rack in preheated dH2O at 55°C for <1 minute and carefully remove coverslips.

Immerse slides in preheated Wash Solution at 55°C and carefully remove the coverslips.

Coverstips slide off by gently agitating the slide rack in Wash Solution. Occasionally, coverstips must be pushed off.

### Wash and Mount
- **Water Rinse: GNRs only**
- **Remove coverslips**
- **Stringent Wash**
- **Mount**

Immerse slides in preheated Wash Solution at 55°C and carefully remove the coverslips.

Incubate for 30 minutes at 55 ± 1°C Remove slide rack and allow slides to air dry.

Add one drop of Mounting Medium to the smear. Add coverseil. Avoid air bubbles.

Examine slides on fluorescence microscope using 60x or 100x oil objective.

### Examine

Examine slides on fluorescence microscope using 60x or 100x oil objective.

*Refer to PNA FISH package insert for complete instructions for use.*
PNA FISH Procedure

1) Fix
   1 drop Fixation Solution
   1 drop + Blood Culture
   Fix cells by heat, flame OR methanol fixation
   Immerse in Ethanol for 5 min.

2) Hybridize
   1 drop of PNA Probe and a coverslip
   Hybridize at 55 °C for 90 min.
   Prepare and pre-heat Wash Solution to 55 °C

3) Wash & Mount
   Immerse in Wash Solution and remove coverslip
   Wash at 55 °C for 30 min.
   1 drop of Mounting Medium and a coverslip

4) Examine
   Examine using Fluorescence Microscope with 60x or 100x oil objective
Multicenter Evaluation of a New Shortened Peptide Nucleic Acid Fluorescence *In Situ* Hybridization Procedure for Species Identification of Select Gram-Negative Bacilli from Blood Cultures

Margie Morgan, Elizabeth Marlowe, Phyllis Della-Latta, Hossein Salimnia, Susan Novak-Weckley, Fann Wu, and Benjamin S. Crystal

### Table 1. Performance of shortened *E. coli/P. aeruginosa* PNA FISH versus routine laboratory identification methods and standard PNA FISH

<table>
<thead>
<tr>
<th>Study site</th>
<th>Sensitivity for <em>E. coli</em></th>
<th>Sensitivity for <em>P. aeruginosa</em></th>
<th>Specificity</th>
<th>Blood culture system used</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Routine</td>
<td>Std</td>
<td>Routine</td>
<td>Std</td>
</tr>
<tr>
<td>A</td>
<td>51/51</td>
<td>51/51</td>
<td>12/12</td>
<td>12/12</td>
</tr>
<tr>
<td>B</td>
<td>51/51</td>
<td>51/51</td>
<td>9/9</td>
<td>9/9</td>
</tr>
<tr>
<td>C</td>
<td>17/17</td>
<td>17/17</td>
<td>7/7</td>
<td>7/7</td>
</tr>
<tr>
<td>D</td>
<td>32/32</td>
<td>32/32</td>
<td>7.8%</td>
<td>7.7</td>
</tr>
<tr>
<td>Total (n = 368)</td>
<td>100% (151/151)</td>
<td>100% (151/151)</td>
<td>97.2% (35/36)</td>
<td>97.2% (35/36)</td>
</tr>
</tbody>
</table>

95% CI

---

*a* Std, standard PNA FISH procedure.

*b* One sample was identified as a mixed *P. aeruginosa/VRE* culture by routine methods and was negative for both the rapid and standard procedures.
Multicenter Evaluation of the Candida albicans/Candida glabrata Peptide Nucleic Acid Fluorescent In Situ Hybridization Method for Simultaneous Dual-Color Identification of C. albicans and C. glabrata Directly from Blood Culture Bottles\(^7\)

Janeen R. Shepard,\(^1\) Rachel M. Addison,\(^7\) Barbara D. Alexander,\(^7\) Phyllis Della-Latta,\(^2\) Michael Gherna,\(^2\) Gerhard Hause,\(^5\) Gerri Hall,\(^4\) Jennifer K. Johnson,\(^3\) William G. Merz,\(^5\) Heidrun Peltroche-Llaesahuanga,\(^5\) Henrik Stender,\(^1\) Richard A. Venezia,\(^2\) Deborah Wilson,\(^6\) Gary W. Procop,\(^6\) Fann Wu,\(^4\) and Mark J. Fiandaca\(^1\)

![Image](image.png)

FIG. 1. Smears made from blood culture bottles spiked with C. albicans (A), C. glabrata (B), both C. albicans and C. glabrata (C), and S. cerevisiae (D) tested with the C. albicans/C. glabrata PNA FISH method. All cultures grown for 6 h at 37°C in BacT/ALERT FN medium (bioMérieux, Durham, NC).

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Blood culture system(s)</th>
<th>Sensitivity</th>
<th>% (no./total)</th>
<th>PPV</th>
<th>NPV</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C. albicans</td>
<td>C. glabrata</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>BacT/Alert, BACTEC</td>
<td>100 (10/10)</td>
<td>100 (5/5)</td>
<td>100 (15/15)</td>
<td>100 (3/3)</td>
<td>100 (3/3)</td>
</tr>
<tr>
<td>B</td>
<td>BACTEC</td>
<td>100 (25/25)</td>
<td>100 (5/5)</td>
<td>100 (30/30)</td>
<td>100 (27/27)</td>
<td>100 (27/27)</td>
</tr>
<tr>
<td>C</td>
<td>BacT/Alert</td>
<td>92.30 (12/13)</td>
<td>100 (3/3)</td>
<td>100 (5/5)</td>
<td>90.0 (9/10)</td>
<td>100 (10/10)</td>
</tr>
<tr>
<td>D</td>
<td>BacT/Alert</td>
<td>100 (17/17)</td>
<td>100 (11/11)</td>
<td>100 (28/28)</td>
<td>100 (25/25)</td>
<td>100 (25/25)</td>
</tr>
<tr>
<td>E</td>
<td>BacT/Alert, BACTEC 9240</td>
<td>100 (14/14)</td>
<td>100 (13/13)</td>
<td>100 (27/27)</td>
<td>100 (17/17)</td>
<td>100 (17/17)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>98.7 (78/79)</td>
<td>100 (37/37)</td>
<td>100 (115/115)</td>
<td>98.8 (81/82)</td>
<td>100 (82/82)</td>
</tr>
</tbody>
</table>
Panel: Bacteria and antibacterial resistance identified by the Prove-it sepsis assay

Gram-negative species
- Neisseria meningitidis
- Enterobacter aerogenes
- Enterobacter cloacae
- Escherichia coli
- Klebsiella oxytoca
- Klebsiella pneumoniae
- Proteus mirabilis
- Proteus vulgaris
- Salmonella enterica subspecies enterica*
- Serratia marcescens
- Enterobacteriaceae family†
- Acinetobacter baumannii
- Pseudomonas aeruginosa
- Stenotrophomonas maltophilia
- Haemophilus influenzae
- Campylobacter jejuni and Campylobacter coli
- Bacteroides fragilis group†

Gram-positive species
- Staphylococcus aureus
- Staphylococcus epidermidis
- Coagulase-negative staphylococcus§
- Streptococcus pyogenes
- Streptococcus agalactiae
- Streptococcus dysgalactiae subspecies equisimilis
- Streptococcus pneumoniae
- Enterococcus faecalis
- Enterococcus faecium
- Listeria monocytogenes
- Clostridium perfringens

Antibacterial resistance
- Meticillin resistance marker mecA

* Covers the serovars Enteritidis, Oranienburg, Othmarschen, Paratyphi, Stanley, Typhi, Typhimurium, Virchow, and groups A, B, C, and D.† Covers the species Citrobacter freundii, Citrobacter koseri, Citrobacter braakii, Enterobacter hormaechei, Enterobacter sakazaki, Klyuyvera intermedia, Morganella morganii, Proteus agglomerans, Providencia rettgeri, Providencia stuartii, Yersinia enterocolitica, and Yersinia pseudotuberculosis.§ Covers the species B fragilis and Bacteroides vulgatus.§ Covers the species Staphylococcus capitis, Staphylococcus lugdunensis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus saprophyticus, Staphylococcus warneri, and Staphylococcus xylosus.
Accurate and rapid identification of bacterial species from positive blood cultures with a DNA-based microarray platform: an observational study

Paivi Tissari, Alimuddin Zumla, Eveliina Tarja, Sointu Meora, Laura Savolainen, Martti Vaara, Anne Aittakorpi, Sanna Laakso, Merja Lindfors, Heli Piparinen, Minna Maki, Caroline Corder, Jim Huggett, Vanya Gant

2107 positive blood culture samples; 0.5ml sample, DNA extracted by automated method followed by multiplex PCR

- Amplification of gyrA, parE and mecA
- 86% of positive cultures had an organism included in the microarray
- Clinical sensitivity 94.7%; specificity 98.8%
- Results in median of 18 hours faster than conventional methods
- Microarray processing time = 3 hours
  - PCR, 1.5 h; Hybridization, 1h; Detection, 1 minute

Addendum: Now over 3300 samples analyzed
FilmArray Blood Culture Panel

**Bacteria:**
- S. aureus
- H. influenzae
- N. meningitidis
- S. pyogenes
- S. agalactiae
- S. pneumoniae
- P. aeruginosa
- E. coli
- A. baumannii
- Coagulase Neg. Staph.
- Enterococcus spp.
- Streptococcus spp
- Enterobacter spp.
- Pan Enterobacteriaceae
- K. pneumoniae
- K. oxytoca

**Antibiotic Resistance:**
- mecA
- van ABC
- blaKPC
- blaTEM
- blaSHV
- blaCTX-M
- blaOXA-58

**Fungi:**
- C. albicans
- Candida spp.

 ASM General Machine

Courtesy of Wade Stev
Laboratory Acceptance of Molecular Methods

- Molecular technologies hold great promise but there are some barriers to laboratory adoption of the technology.
- Molecular methods are expensive and microbiologists often need to make a business case to hospital administration to purchase them.

- Cost savings due to rapid detection of organisms and resistance may be greater outside of the laboratory than in (reduced pharmacy costs, reduced length of stay, fewer laboratory and other diagnostic tests ordered).
- For many specimen types, molecular assays do not replace traditional cultures but augment them.
- “Is the juice worth the squeeze?”
CONCLUSIONS

- Traditional culture and susceptibility testing methods are too slow to truly impact the appropriate treatment of sepsis and improve antimicrobial use.
  - Identifying organisms and generating AST results using traditional methods have clinical value, but the value can be greatly improved by decreasing turn around time.

- Molecular methods can inform therapeutic decisions early in the course of therapy, particularly when resistant organisms can be identified.

- Physician acceptance of novel methods may require considerable resources and can be a barrier to implementation.

- None-the-less, implementing molecular methods for rapid diagnosis can result in significant savings for the hospital in addition to improving patient outcomes.