Comparison of peptide nucleic acid fluorescence *in situ* hybridization assays with culture-based matrix-assisted laser desorption/ionization-time of flight mass spectrometry for the identification of bacteria and yeasts from blood cultures and cerebrospinal fluid cultures

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Abstract

Peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH) is a molecular diagnostic tool for the rapid detection of pathogens directly from liquid media. The aim of this study was to prospectively evaluate PNA FISH assays in comparison with culture-based matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) identification, as a reference method, for both blood and cerebrospinal fluid (CSF) cultures, during a 1-year investigation. On the basis of the Gram stain microscopy results, four different PNA FISH commercially available assays were used ('*Staphylococcus aureus*/CNS', '*Enterococcus faecalis*/OE', 'GNR Traffic Light' and 'Yeasts Traffic Light' PNA FISH assays, AdvanDx). The four PNA FISH assays were applied to 956 positive blood cultures (921 for bacteria and 35 for yeasts) and 11 CSF cultures. Among the 921 blood samples positive for bacteria, PNA FISH gave concordant results with MALDI-TOF MS in 908/921 (98.64%) samples, showing an agreement of 99.4% in the case of monomicrobial infections. As regards yeasts, the PNA FISH assay showed a 100% agreement with the result obtained by MALDI-TOF MS. When PNA FISH assays were tested on the 11 CSF cultures, the results agreed with the reference method in all cases (100%). PNA FISH assays provided species identification at least one work-day before the MALDI-TOF MS culture-based identification. PNA FISH assays showed an excellent efficacy in the prompt identification of main pathogens, yielding a significant reduction in reporting time and leading to more appropriate patient management and therapy in cases of sepsis and severe infections.

Keywords: Bacteraemia, blood culture, cerebrospinal fluid, fungaemia, matrix-assisted laser desorption/ionization-time of flight, peptide nucleic acid fluorescence *in situ* hybridization

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Introduction

Rapid detection and identification of microorganisms in blood specimens have been advocated in order to shorten the turnaround time (TAT) for appropriate management of patients suffering from bacteraemia and fungaemia [1–3]. While the automated, continuously monitoring blood culture (BC) systems have reduced the delay in detecting the presence of blood-borne bacteria and fungi, identification of such microorganisms still requires subculturing the microorganism onto solid media [4,5]. At present, different techniques for microorganism identification from positive BCs, such as real-time PCR, DNA microarrays, matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) and peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH), are available to shorten the TAT for the detection of Gram-positive cocci (GPC), Gram-negative rods (GNR) and *Candida* spp. [6–10].

Peptide nucleic acid fluorescence *in situ* hybridization is a molecular technology that rapidly identifies directly from liquid cultures the bacteria and yeasts most frequently responsible for bloodstream infections [7,11,12]. In recent years the MALDI-TOF MS has emerged as a powerful tool for microbial species identification after they have grown on solid media [13–17].

To date, studies utilizing PNA FISH assays have been reported for the detection of GPC, *Candida* spp. and GNR, separately [1,18–22] or in comparison with standard culture methods [7]. The aim of the present study was to prospectively compare PNA FISH assays with culture-based MALDI-TOF MS for the identification of bacteria and yeasts in positive BCs in a clinical routine setting. The second aim was to evaluate the identification accuracy of the PNA FISH assays from cerebrospinal fluids (CSF) inoculated into BC media.

Materials and Methods

Patients and clinical samples

From January to December 2012, 12 051 BCs collected from 4713 patients (2104 female and 2609 male; median age 68 years, range 1 day–101 years) with clinical suspicion of sepsis or other severe infections, such as endocarditis, pneumonia or meningitis, were routinely sent to the Bacteriology Section of the Unit of Clinical Microbiology of the University Hospital of Parma for the diagnosis of bacterial infections. Among the 1907 positive BCs (belonging to 1114 patients), 921 samples (belonging to 886 patients) were selected for the PNA FISH assays. The PNA FISH assays were generally performed on the first sample of each patient; they were eventually repeated in samples of the same patient following the first one if the Gram stain result was different from that of the first sample or in samples collected at least 15 days from the first one. This was in order to check whether the same or a new microorganism was involved in a second episode of sepsis.

If a set of BCs from a patient (aerobic plus anaerobic bottles) was positive, a single bottle was evaluated (the first that tested positive).

In the same period, 35 (belonging to 34 patients) of the 138 positive samples (belonging to 55 patients) were selected for *Candida* spp. PNA FISH assay, following the same criteria as for bacteria. The 138 positive samples were obtained from 3740 blood samples collected from 1194 patients (544 female and 650 male; median age 68 years, range 1 day–99 years) with clinical suspicion of fungaemia and routinely sent to the Mycology Section of the above-mentioned Unit of Clinical Microbiology.

Among the 410 samples of CSF (belonging to 197 patients; 88 female and 109 male; median age 49 years, range 1 day–91 years) routinely examined during 2012, a panel of 11 positive BC bottles inoculated with CSF (belonging to 11 patients) was selected, as described above for bacterial BCs, for the PNA FISH assays from 45 positive samples (belonging to 27 patients). These patients presented with hydrocephalus, clinical suspicion of central nervous system infection (meningitis and encephalitis) or polytrauma involving the central nervous system.

The samples analysed in this study were sent to the University Hospital of Parma for routine diagnostic purposes, and the laboratory diagnosis results were reported in the medical records of the patients as a diagnostic answer to a clinical suspicion of sepsis; ethical approval at the University Hospital of Parma is required only in cases in which the clinical samples are to be used for applications other than diagnosis.

Conventional blood/cerebrospinal fluid culture processing and reference method for identification from solid culture

Blood and CSF cultures were screened for microbial growth with the Bactec FX system (Becton Dickinson, Sparks, MD, USA), using BACTEC Plus Aerobic F, Plus Anaerobic F, Plus Ped and Mycosis IC/F bottles (Becton Dickinson) according to standard methods and to the manufacturer's recommendations [23]. As part of a routine culture set for bacterial detection, 8–10 mL of blood were inoculated into each aerobic and anaerobic bottle, and incubated at 37°C; for yeast detection Mycosis IC/F bottles were used. In the case of CSF samples, in parallel with conventional culture on solid media, an aliquot (1–3 mL) was inoculated into a Bactec Plus Ped bottle. When a bottle signalled positive by the Bactec FX instrument a Gram stain was performed and an aliquot was subcultured onto blood agar, MacConkey agar, Schaedler agar and/or Sabouraud dextrose agar with chloramphenicol (KIMA, Piove di Sacco-PD, Italy) and incubated at 37°C in the appropriate conditions according to bottle type and Gram staining result. After sufficient (at least two to three colonies) pure microbial growth was achieved (24–72 h), identification tests were performed by a Microflex LT- MALDI Biotyper mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany, supplied by Becton Dickinson, Italy) in the case of Gram-negative bacteria and by a VITEK MS mass spectrometer (bioMérieux, Marcy L'Etoile, France) in the case of Gram-positive bacteria and yeasts, according to the laboratory workflow.

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry was performed according to the manufacturer's instructions. A single bacterial colony of fresh overnight