A novel fluorescence in situ hybridization test for rapid pathogen identification in positive blood cultures

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Abstract

A novel molecular beacon-based fluorescence in situ hybridization (FISH) test allowing for the identification of a wide range of bacterial pathogens directly in positive blood cultures (BCs) was evaluated with positive BCs of 152 patients. Depending on the Gram stain, either a Gram-negative or a Gram-positive panel was used. The time to result was 30 min, and the hands-on time was only 10 min. Seven per cent of the cultured microorganisms were not included in the FISH panels; the identification rate of those included was 95.2%. Overall, the FISH test enabled accurate pathogen identification in 88.2% of all cases analysed.

Keywords: Blood culture, fluorescence in situ hybridization, identification, pathogen

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Blood culture (BC) still represents the reference standard for the detection and identification of bloodstream pathogens [1]. In routine clinical practice, as soon as growth is detected in a BC bottle, a Gram stain is performed [2]. With conventional microbiological techniques, it usually takes several hours or even days from the time of microscopy to definite pathogen identification [2]. On the other hand, the importance of rapid diagnosis of sepsis and septic shock allowing for adequate therapeutic measures such as the initiation or adjustment of a targeted treatment has been well documented [3,4]. Apart from molecular technologies for the detection of pathogen DNA directly from whole blood specimens [5–7], laboratory methods accelerating the definite identification of microorganisms grown in BCs are also of crucial importance, and have been the subject of intensive research and development during recent years [8–16].

Today, in addition to a multitude of home-made molecular assays, several commercially available tests already exist, allowing for a culture-independent identification of pathogens grown in BCs. Molecular tests based on multiplex PCR accurately detect bloodstream pathogens accounting for >90% of sepsis episodes; moreover, detection of important resistance markers, such as those for methicillin in staphylococci and those for vancomycin in enterococci, may also be feasible [8,9]. However, considering the time to result of >4 h, the prolonged hands-on time, and the high risk of DNA contamination, the usefulness of these tests in daily laboratory routine may be questionable. With due consideration of time and cost savings, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), e.g. with the Sepsityper kit (Bruker Daltonics, Bremen, Germany), may represent the method of choice allowing for direct pathogen identification in >80% of positive BCs [10]; the main drawbacks of this method are the cost of acquisition of the system, meaning that it is affordable only for larger laboratories, and the exclusive assignment for research use. Other tests for rapid isolate identification in positive BCs, such as the tube coagulase test, Staphylococcus aureus real-time PCR, including detection of methicillin resistance, antigen detection (e.g. for Streptococcus pneumoniae), and molecular fluorescence in situ hybridization (FISH) assays, are appropriate for integration into daily routine analysis [11–16]; however, they are only capable of detecting either a single pathogen or a limited number of microorganisms.

Here, we evaluated a novel commercially available FISH assay (hemoFISH; Miacom Diagnostics, Düsseldorf, Germany) allowing for the direct identification of a wide range of Gram-positive and Gram-negative bloodstream pathogens in positive BCs accounting for more than 90% and 80% of cases, respectively (Fig. 1) [17,18]. The test uses molecular beacons as DNA probes binding to the rRNA of microbial ribosomes. Analysis was conducted during a period of three months. One positive BC per patient of consecutive patients was analysed by FISH with the bottle (aerobic FA or anaerobic FN) becoming positive first with the BacT ALERT 3D system (BioMerieux, Marcy l’Etoile, France). In the case of neonates and infants, only the paediatric PF bottle was available. Samples were stored at room temperature until FISH analysis, which was performed once daily. The test was performed by an experienced technician, who was aware only of the Gram stain result.
Depending on the latter, either a Gram-negative or a Gram-positive panel was used (Fig. 1), as recommended by the manufacturer. Briefly, 10 lL of a 1/20 dilution of a BC aliquot was applied to each of the eight fields on a microscopy slide. After being dried on a heat block, bacterial cells were perforated with lysis buffer and fixed in an ethanol bath, prior to a 10-min hybridization step with the fluorescent DNA probes. After termination of the reaction by briefly dipping the slide in a stop buffer, mounting medium was added, and reading was performed on a fluorescence microscope (Olympus BX41; Olympus Deutschland, Hamburg, Germany) equipped with rhodamine and fluorescein filters. The time to result was 30 min, and the hands-on time only 10 min.

As for Enterobacteriaceae, fluorescence should not be limited to the species-specific field, if available, but should also include the field of the family-specific probe. Similarly, the field of the respective genus-specific probe should be also positive with Staphylococcus aureus or Streptococcus pneumoniae, Streptococcus agalactiae, and Streptococcus pyogenes (Fig. 1). Subcultured isolates were routinely identified to the species level by MALDI-TOF MS (microflex; Bruker Daltonics). When MALDI-TOF MS identification failed or was not certain, molecular identification with a broad-range PCR and sequence-analysis protocol was performed, as described previously [19]. Streptococcus pneumoniae isolates were always confirmed by optochin testing. This study was approved by the institutional review board at the Medical University Vienna.

During the evaluation period, 157 bacterial isolates (59 Gram-negatives and 98 Gram-positives) were identified from 152 positive BCs analysed by FISH. Eleven of 157 microorganisms (7%) were not included in the FISH panels, and in one case staphylococci were not detected, because only the Gram-negative panel was used, owing to overgrowth by Bacteroides stercoris (Table 1). Of the remaining 145 microorganisms, 138 were identified correctly, three were misidentified, two were identified to the family but not to the species level, although the species-specific probes were included in the panel, and one was not identified at all by FISH (Table 1). In only one case of Enterococcus faecalis monoculture, fluorescence was also detected with the specific DNA probe for Streptococcus agalactiae (but not with that for the genus Streptococcus), suggesting cross-reactivity (Table 1). In cases of discrepancy between FISH and the result obtained routinely, the latter was confirmed by molecular analysis of the respective isolate. Overall, the FISH test identification rate for microorganisms contained in the FISH panels was 95.2% (138/145; 95% CI 90.3–98%), suggesting cross-reactivity (Table 1). In cases of discrepancy between FISH and the result obtained routinely, the latter was confirmed by molecular analysis of the respective isolate. Overall, the FISH test identification rate for microorganisms contained in the FISH panels was 95.2% (138/145; 95% CI 90.3–98%). The identification rates with the Gram-negative and Gram-positive panels were 92.7% (51/55; 95% CI 82.4–98%) and 96.7% (87/90; 95% CI 90.6–99.3%), respectively. There was no appreciable difference in test performance between aerobic and anaerobic BC bottles (data not shown). The somewhat lower performance of the Gram-negative panel was solely attributable to incorrect or insufficient identification of some isolates of the genus Klebsiella. Beyond that, it would be useful if the Gram-negative panel also allowed for the detection of the genera Acinetobacter and Enterobacter, regarding the latter genus, however, an isolate belonging to the family Enterobacteriaceae, without belonging to any of the species identified by this panel, is very likely to produce chromosomal AmpC β-lactamases, and thus shows a predictable resistance pattern [20].

Overall, the FISH test enabled accurate pathogen identification in 88.2% (134/152) of all analysed cases of bacterial growth in the BC. We believe that, because of the fast and
flexible analytical procedure, this test can be integrated into the daily routine and repeatedly performed during the day for early BC diagnostic purposes. Furthermore, it may represent a useful complementary test to MALDI-TOF MS if the latter is used for direct pathogen identification in positive BCs.

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Transparency Declaration

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References