Rapid Identification of Pathogens in Blood Cultures with a Modified Fluorescence In Situ Hybridization Assay

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We evaluated a modified fluorescence in situ hybridization (FISH) assay for rapid (<1 h) identification of microorganisms in growth-positive blood cultures. The results were compared to those of the standard FISH technique and conventional culturing. The rapid identification of microorganisms with modified FISH can have important effects on clinical management of patients with bloodstream infections.

Blood culturing is the “gold standard” for diagnosis of bloodstream infections (BSI). Results of microbiological culturing can take up to several days, which may be too slow for a patient with a quickly deteriorating sepsis (12). Therefore, techniques that speed up the diagnosis of BSI would be most useful.

Several molecular techniques that permit faster identification of microorganisms in growth-positive blood cultures have become available, including fluorescent in situ hybridization (FISH) (8, 9, 14). The standard FISH technique requires approximately 4 h to identification in routine practice (13).

In the present study we evaluated a new, rapid FISH procedure, modified to permit the identification of microorganisms in blood cultures within 1 h after Gram stain results.

Study set-up. We included 100 consecutive growth-positive blood cultures from febrile patients admitted to our academic hospital. The present study was carried out partially in parallel with another study in which the time to identification by standard FISH was evaluated (13). Ninety-four blood cultures were included in both studies; another 6 were included in the present study, resulting in a total of 100 samples.

Blood cultures were included upon detection of growth by the blood culture machine (BACTEC 9240). Gram staining of the blood culture fluid was performed, and only the first positive blood culture from a single patient was included, except when different Gram stain characteristics were observed in subsequent blood cultures. Aliquots of blood culture fluid were used for standard and modified FISH and for routine identification by standard microbiological techniques.

FISH. The standard and modified FISH assays were performed with the seaFAST Sepsis Kit (SeaPro International B.V., Lelystad, The Netherlands), according the manufacturer’s protocols. Blood culture fluid was centrifuged at 1,200 rpm (∼450 × g) for 1 min, and the supernatant fraction was applied to a glass slide. Slides with gram-positive microorganisms were incubated in a lysis mixture, followed by an ethanol bath for all slides for permeabilization. Tandem oligonucleotide probes were applied and hybridized to target rRNA (Table 1). Unbound probe was washed off, and the slides were read by fluorescence microscopy (Olympus BX40, 100×).

The modified FISH assay had two modifications from the standard protocol. First, nisin was added to the lysis mixture. Nisin is a lantibiotic peptide that forms pores in bacterial cell walls (6). Second, the hybridization and washing steps were done in a microwave oven instead of in a water bath. The hybridization chamber was placed in a plastic box with water preheated to 46°C. This box was then placed in the microwave (SHARP R208) and heated to 48°C, after which cooling down to 46°C was allowed. This 4.5-min cycle was done four times for hybridization and three times for washing. The turnaround time of this modified procedure was less than 1 h.

Sequence analysis. Sequence analysis of the DNA of microorganisms from all samples with results that were discrepant between FISH and microbiological culturing was performed. After DNA isolation (QIAamp Mini Kit; QIAGEN), 16S rRNA gene amplification was done, followed by sequence determination with an ABI Prism 3100 genetic analyzer (Applied Biosystems), according to the manufacturer’s instructions.

Results. The genus of the gram-positive microorganisms tested was identified correctly by modified FISH in 96% of cases, whereas the family name was identified in 69% of gram-negative microorganisms (Table 2). The species name could be attributed to 81% of all microorganisms, and eubacterial and pan-yeast probes were positive for 100% of the relevant samples. There was only one discrepant case between modified and standard FISH. One strain of cultured Staphylococcus aureus (as confirmed by sequence analysis) was identified correctly with modified FISH, but in standard FISH, fluorescence was observed only with the Staphylococcus genus probe.
The sensitivities of the Enterobacteriaceae- and genus-specific probes were 96% and 100%, respectively. Also, the sensitivities of the species-specific probes for microorganisms encountered in our study were 100%, except for the Escherichia coli probe (14 of 15): for one isolate of cultured Escherichia coli, the abundance of blood cells and protein made it impossible to distinguish background fluorescence from fluorescent bacteria with the Escherichia coli and the Enterobacteriaceae probes.

Identification by FISH was incorrect in two cases: fluorescence was observed with the Klebsiella pneumoniae probe for one isolate of cultured Klebsiella oxytoca, as confirmed by sequence analysis. Alignment of the 23S rRNA sequence of Klebsiella oxytoca with the Klebsiella pneumoniae probe revealed two mismatches. FISH was not performed on the strain isolated in culture. In another sample, fluorescence was observed with the Haemophilus influenzae probe, but only non-fermenting gram-negative rods, which were identified as Roseomonas species by sequence analysis, were cultured (13).

**Discussion.** We show that a modified FISH assay can identify the vast majority of microorganisms in blood cultures within 1 h. The performance of this assay was similar to the standard FISH technique and even identified Staphylococcus aureus in an extra case. To our knowledge, the use of a microwave oven for hybridization of DNA probes in FISH has been described for chromosome analysis but not for identification of bacteria (3, 5). We observed a subjectively higher intensity of fluorescence with microwave-based FISH, which was also described previously (5).

The diagnostic spectrum of the FISH assay should be further enhanced by the inclusion of more probes; in particular, extra probes targeting gram-negative microorganisms would be very useful. The false-positive FISH result that we observed with the Klebsiella pneumoniae probe for the cultured Klebsiella oxytoca may signal a problem with the specificity of this probe.
The turnaround time of the modified FISH assay was less than 60 min, which is a major time gain compared to standard FISH. This short turnaround time can have great impact on the management of patients with BSI (Fig. 1). Most therapeutic interventions occur after phlebotomy and upon notification of Gram stain results from blood culture fluids (11). With the identification of microorganisms within 1 h of Gram staining, it may become feasible for microbiologists to wait for FISH identification rather than providing the clinician with only Gram stain results.

In conclusion, the modified FISH assay appears to be a promising tool for the early optimization of antimicrobial therapy for patients with BSI.

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REFERENCES