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LightCycler® SeptiFast Test: rapid detection of nosocomial pathogens by real-time PCR

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Objective: At present, one of the main medical problems in hospitals is the increasing number of nosocomial (hospital-acquired) infections. Epidemiological data show that a limited number of microorganisms are responsible for the majority of bloodstream infections. Twenty-five species account for more than 90% of all nosocomial pathogens. A rapid diagnosis of Sepsis and the correct identification of the causative pathogen followed by immediate and appropriate antimicrobial therapy is the key of success in the management of bacteraemia and fungaemia in septic patients, but not limited to these.

Methods: The LightCycler® SeptiFast Test was developed to detect and differentiate up to 25 pathogenic microbial DNA(s) in human whole blood. Specimen preparation is based on a semi automated procedure using the MagNa Lyser followed by a manual spin column based nucleic acid preparation. Amplification and detection are automated using the LightCycler 2.0 Instrument.

Results: Compared to classical methods (blood culture followed by gram staining and species identification based on culture methods), the LightCycler® SeptiFast Test offers an improved sensitivity and a much better time to result. In most cases, the result will be available in about 4.5 hours instead of two to three days. In case of a positive PCR result, therapy can be adjusted about two days earlier, which might lead to significant savings for the hospital as well as to improved outcome for the patient. In case of negative PCR results, blood culture remains the basis for medical decisions.

Conclusion: The LightCycler® SeptiFast Test is the first IVD assay using PCR for the rapid detection and identification of bacterial and fungal pathogens involved in nosocomial infections directly from whole blood. Here we present non-clinical performance data as well as selected data from several external clinical studies to illustrate the ability and power of the assay.
LightCycler® SeptiFast Test m: Rapid Detection of Nosocomial Microorganisms by Real-Time PCR
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Abstract
At present, one of the main medical problems in hospitals is the increasing number of nosocomial (hospital-acquired) infections. Epidemiological data show that a limited number of microorganisms are responsible for the majority of bloodstream infections. Twenty-five species account for approx. 80% of all nosocomial pathogens.
A rapid diagnosis of sepsis and the correct identification of the causative pathogen followed by immediate and appropriate antimicrobial therapy is the key to success in the management of sepsis and septicus e.g. in septic patients.
The LightCycler® SeptiFast Test m was developed to detect and differentiate up to 25 microorganisms causing bacterial/fungal blood stream infections in human whole blood. Specimen preparation is based on a semi-automated procedure using the MagnaLyser® Instrument followed by a manual spin column based nucleic acid preparation. Amplification and detection are achieved by the LightCycler® 2.0 Instrument.
Compared to classical methods (bacterial culture followed by gram staining and species identification based on culture methods), the LightCycler® SeptiFast Test m offers a higher positive rate and a much faster time to result. In most cases, the result will be available in less than 6 h.
The LightCycler® SeptiFast Test m is the first IC assay using PCR for the rapid detection and identification of bacterial and fungal microorganisms involved in nosocomial infections directly from whole blood. Here we present non-clinical performance data as well as selected data from several external clinical studies to illustrate the ability and power of the assay.

Summary
• The LightCycler® SeptiFast Test m presented here is the first IC assay for rapid detection and differentiation of bacterial and fungal microorganisms involved in nosocomial infections by real-time, on-line PCR analysis (Fig. 1).
• The assay allows detection down to 25 Gram- (+), Gram- (-) and fungal microorganisms that can be differentiated into 20 different groups of microorganisms.
• Specimen preparation is based on a semi-automated, spin column based procedure directly from K-EDTA blood samples, followed by a color, real-time PCR amplification for the specific detection and differentiation of amplicons by melting curve analysis using the LightCycler® 2.0 Instrument (mK +).
• Assay-specific internal and external controls are detected simultaneously to the target nucleic acids and in the same PCR reaction and run respectively to allow to detect the complete nucleic acid isolation/amplification process. Moreover interpretation of false-negative results is eliminated (Fig. 1 and 2).
• Currently available commercial reagents contain significant amounts of nucleotide analogues which interfere with the detection of bacterial/fungal DNA using the LightCycler® SeptiFast Test m.
• The analytical sensitivity of the LightCycler® SeptiFast Test m was analyzed by hit-rate analysis of each analyte. A minimum sensitivity of 30 CFU/ml was observed for all species, except for Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus pyogenes and C. glabrata (100 CFU/ml) (data not shown).
• To prove the uniformity and homogeneity of the internal transcribed spacer (ITS) target region, clinical isolates originating from several geographical regions in Europe (southern, middle, northern) and US were collected. In total 158 isolates were characterized by microbological methods and tested with LightCycler® SeptiFast Test m.
• 98.7% of all samples (158/152) were detected by both methods with an overall accuracy of 98.9% (= number of correctly detected isolates / number of isolates detected) (110 / 110) (Fig. 4).
• Cross-check analysis of 31 other, closely related bacterial and fungal microorganisms revealed no cross reactions with the LightCycler® SeptiFast Test m (Fig. 5).
• In addition, analysis of blood samples spiked with commonly used drugs from different substance classes showed no interference with the detection of bacterial and fungal DNA by the LightCycler® SeptiFast Test m (Fig. 6).
• Clinical performance of the assay has been established in an in-vitro study including 217 patient (K blood samples) using a center study involving 4 European sites including 276 (ICU) patients with suspected sepsis and other bacterial/fungal blood stream infections (728 blood samples, data not shown).
• Important findings of the clinical performance of the LightCycler® SeptiFast Test m were:
• Analysis of patients without suspicion for sepsis or blood stream infections revealed a diagnostic specificity of 99.1%.
• During PCR, amplicons were simultaneously detected by fluorescence using different Hybridization Probes specific for the target DNA- and the IC-derived amplicons respectively (4-color detection).
• Preparation of bacterial and fungal nucleic acids
Microorganisms isolated from blood were prepared by artificially spiking of negative whole blood with quantified cryoprecipitates of the target organisms present in nosocomial bloodstream infections. Concentration of cryoprecipitates were determined by total cell counts and colony forming units (CFU).
In addition to spiked material, characterized clinical isolates (K-EDTA blood samples) from individuals with suspected sepsis and other bacterial/fungal blood stream infections were used.

Results

Fig. 1. LightCycler® SeptiFast Test m – Sample testing flow chart.

Fig. 2. Channel distribution exemplified for Gram (+) differentiating PCR.

Fig. 3. m quality of LightCycler® SeptiFast reagents. Several dilutions of A. naeem spir, were analyzed by commercial quality (A) and DNA-verified (B) PCR reagents in 3x, calf-spleen PCR reagent.

Fig. 4. Environmental interference. The nucleic acid based IC does not interfere with the detection of bacterial and fungal DNA with the LightCycler® SeptiFast Test m.

Materials and Methods
Sample material
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