

PCR Detection of Sepsis-Inducing Pathogens in Blood Using SepsiTTM

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Introduction

PCR enables the identification of bacterial DNA in culture-negative samples from patients with suspected infection. Goals of PCR diagnosis for pathogens in blood (sepsis) are improved disease surveillance, early guidance on appropriate antibiotic therapy and patient management.

Two new universal rDNA PCR systems (Molzymb, Bremen) for bacteria and yeast pathogen detection are described, **SepsiTTM** and **MolYsis Complete5** plus **Mastermix 16S** (PCR). By sequencing of the amplicon and BLASTN search strains present in the blood sample are identified. Results from an ongoing diagnostic study for bacterial pathogens, using samples from critically ill patients, are presented, comparing the molecular tests among each other and with blood culturing as the reference.

Methods

Samples from SIRS, sepsis or neutropenic fever patients from University Hospital Ulm (group A) and Hospital Cologne (group B) were analysed. The two Real-Time (RT)-PCR systems are comparable in the blood extraction and assay methodologies: Group A samples were analysed using 5 ml blood (**MolYsis Complete5 / Mastermix 16S**) and group B 1 ml blood (**SepsiTTM**). RT-PCR was run on a Opticon DNA Engine (BioRad). Statistical analysis of RT-PCR results was performed against blood culture (BC) results. Identification of strains was done by BLASTN analysis (EMBL/Genbank), including $\geq 94\%$ sequence identity results (genus and species level).

Results

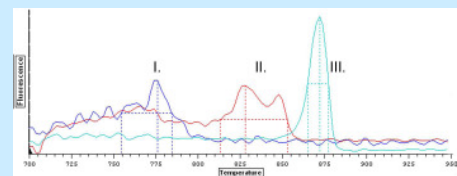


Fig. 1: Melting curve (RT-PCR) analysis of typical samples of negative controls (primer dimers, I.), MolYsis Complete5 extract of negative blood (traces of human DNA, II.) and *S. aureus* DNA (III.). Melting curves of other bacteria tested all peaked at $\geq 87^\circ\text{C}$ (1).

The specific signal (Fig. 1, III.) is clearly separated from the unspecific signals (I. and II.).

References: (1) *Diag. Microbiol. Inf. Dis.* (2008) doi:10.1016/j.diagmicrobio.2008.07.011

Table 1: Pathogens among the >345 species validated by homology search using the set of primers for bacteria resp. yeasts.

Bacteria		Yeasts	
species	count	species	count
Gram-negative		Gram-positive	
<i>Achromobacter</i>	1	<i>Salmonella</i>	2
<i>Acinetobacter</i>	5	<i>Serratia</i>	4
<i>Acinetobacter</i>	2	<i>Shewanella</i>	3
<i>Aeromonas</i>	7	<i>Staphylococcus</i>	1
<i>Aggregatibacter</i>	3	<i>Stenotrophomonas</i>	1
<i>Alcaligenes</i>	2	<i>Vibrio</i>	7
<i>Alistipes</i>	1	<i>Xanthomonas</i>	1
<i>Anaplasma</i>	1	<i>Yersinia</i>	2
<i>Bacteroides</i>	2		
<i>Bifidobacterium</i>	9	Sum:	114
<i>Brevibacterium</i>	1		
<i>Brevitimonas</i>	2		
<i>Burkholderia</i>	6		
<i>Butyrivibrio</i>	1	<i>Achromyces</i>	1
<i>Campylobacter</i>	2	<i>Aspergillus</i>	1
<i>Chlamydia</i>	2	<i>Arthrospira</i>	1
<i>Citrobacter</i>	1	<i>Bacillus</i>	20
<i>Citromonas</i>	1	<i>Clostridium</i>	2
<i>Citrospira</i>	1	<i>Corynebacterium</i>	2
<i>Clavibacter</i>	2	<i>Enterococcus</i>	7
<i>Clavibacter</i>	6	<i>Erysipelothrix</i>	1
<i>Escherichia</i>	1	<i>Exiguobacterium</i>	1
<i>Escherichia</i>	3	<i>Glaciobacterium</i>	2
<i>Haemophilus</i>	4	<i>Frankia</i>	1
<i>Haifa</i>	1	<i>Kocuria</i>	1
<i>Haemobacter</i>	1	<i>Lactobacillus</i>	26
<i>Klebsiella</i>	2	<i>Lactococcus</i>	3
<i>Lactobacillus</i>	5	<i>Lactobacillus</i>	1
<i>Legionella</i>	1	<i>Leuconostoc</i>	2
<i>Methylobacterium</i>	1	<i>Microbacterium</i>	2
<i>Moraxella</i>	2	<i>Micromonospora</i>	1
<i>Mycobacterium</i>	1	<i>Mycobacterium</i>	10
<i>Neisseria</i>	2	<i>Nocardia</i>	3
<i>Neisseria</i>	2	<i>Paenibacillus</i>	3
<i>Pantoea</i>	2	<i>Pediococcus</i>	1
<i>Paenibacillus</i>	1	<i>Phyllobacterium</i>	3
<i>Phytomonas</i>	1	<i>Photobacterium</i>	19
<i>Proteus</i>	2	<i>Staphylococcus</i>	6
<i>Pseudomonas</i>	18	<i>Streptococcus</i>	2
<i>Rahnella</i>	1	<i>Weissella</i>	2
<i>Ralstonia</i>	3		
<i>Rhizobium</i>	1	Sum:	128

252 bacteria and 6 yeast pathogens are detectable by the test (Table 1)

In the blood samples, 17 species were identified by PCR and sequencing (Table 2)

Table 2: Bacterial species identified by RT-PCR + sequencing in blood samples ($\geq 94\%$ identity to EMBL)

Strain
<i>S. epidermidis</i>
<i>E. faecalis</i>
<i>E. coli</i>
<i>Propionibacterium acnes</i>
<i>S. aureus</i>
<i>Streptococcus sp.</i>
<i>S. pneumoniae</i>
<i>Petrobacter sp.</i>
<i>Haemophilus sp.</i>
<i>Gemella sp.</i>
<i>Lactobacillus sp.</i>
<i>P. vulgaris</i>
<i>Methylobacterium sp.</i>
<i>E. faecium</i>
<i>S. sanguinis</i>
<i>Fusobacterium</i>
<i>Enterobacter sp.</i>

Table 3: Results of the diagnostic study with blood samples from critically ill patients (PCR assaying for bacteria)

Study	samples	patients	positive results			negative results			sensitivity (%)	specificity (%)	PPV (%)	NPV (%)
			PCR	BC	PCR+BC	PCR	BC	PCR+BC				
Group A	80	70	32	17	15	48	63	46	88.2	73.0	46.9	95.8
Group B	54	43	32	11	10	22	43	21	90.9	48.8	31.3	95.5
All (A/B)	134	113	64	28	25	70	106	67	89.3	63.2	39.1	95.7

Group A: 5 ml blood, group B: 1 ml blood samples; BC: blood culture; PPV: positive predictive value; NPV: negative predictive value

The diagnostic sensitivity was not higher with 5 ml than with 1 ml blood (Table 3), both setups reaching high values (mean of all 89.3 %). Overall, 2.3 times more positives were observed with RT-PCR (positivity rate, 48%) than with BC (21%). Sequencing confirmed the number of RT-PCR-positives.

Species concordant in RT-PCR + sequencing and BC were (no.): *S. epidermidis* (10), *E. coli* (1), *P. acnes* (1), and *E. faecalis* (1), the remaining 12 blood samples (Table 3, all) of the PCR positives comprised multiple strains. The overall rate of multiple strains in blood samples was 56 % of the RT-PCR positives (sequencing analysis).

Because of the high positivity rate of RT-PCR analysis, the specificity and PPV were low, whereas the high NPV value indicated good accordance to BC.

6 RT-PCR + sequencing positive, BC negative samples were confirmed by results of other microbial cultures taken around blood sampling (Table 4).

Table 4: Case studies – Concordance of sequencing and swabs from other sites (BC negative)

Patient	PCR (blood) + swab analysis	material swab
B10	<i>S. aureus</i>	CVC (MRSA)
B16	<i>Propionibacterium acnes</i>	CVC
B49	<i>S. epidermidis</i>	CVC
A35	<i>Gemella/Streptococcus</i>	intraoperative swab
A45	<i>P. vulgaris</i>	intraoperative swab
A48	<i>P. vulgaris</i>	oral swab

CVC: Central venous catheter

Summary

An alignment algorithm indicated that, using the primers, 252 bacterial pathogens can be PCR detected, of which 17 were identified in blood sample analysis. Detection of bacteraemia by RT-PCR of 134 blood samples from critically ill patients proved to be highly sensitive (89.3 %). More samples were positive by RT-PCR than blood culture. Of the RT-PCR positives, BC negatives 6 were confirmed by positive swabs at other sites. Most samples contained multiple strains (RT-PCR + sequencing).