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Oral Session
PCR and other molecular tests directly on blood: what is new?

MAGICPLEX SEPSIS REAL-TIME PCR FOR RAPID DETECTION OF PATHOGENS IN PATIENTS WITH SUSPECTED SEPSIS

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Objectives
Early, accurate administration of antibiotics is crucial for successful outcome of severe sepsis and septic shock. Blood culture (BC) is the 'gold standard' for diagnosing bloodstream infections, but the method is time-consuming and lack in sensitivity. PCR technology enables rapid pathogen identification directly on blood, and has the potential to shorten the time to adequate, targeted antibiotic therapy.
Magicplex Sepsis Real-time PCR (SeeGene) is a multiplex PCR, detecting more than 90 pathogens. The aim of the present study was to evaluate this commercial PCR by comparing it with BC on a large study cohort of unselected patients with suspected sepsis.

Methods
Venous blood was collected in an EDTA tube together with 2 pairs of BC, from 704 patients with suspected sepsis at the Emergency Department, Örebro University Hospital, Sweden, between February 2011 and February 2012. BC bottles were incubated in the Bactec system (Becton Dickinson). DNA was extracted from 1 mL whole blood using MolYsis Blood Pathogen Kit (Molzym) on an Arrow (Nordiag). Pathogen detection was performed using the Magicplex (MP) Sepsis Real-time test and the remaining DNA preparation was thereafter frozen. Samples with positive results for *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae* or *Streptococcus pneumoniae* were further analyzed using species specific inhouse PCR protocols on the stored DNA preparations. In a few cases the frozen volume was insufficient and the PCR assays was thus performed on MP and/or BC positive samples for 25 *S. pneumoniae*, 26 *S. agalactiae*, 50 *S. aureus*, and 65 *E. coli*.

Results
Valid MP results were obtained for 697 blood samples. Excluding samples positive for contaminants, i.e Coagulase-negative staphylococci (n=109), and samples positive in BC for pathogens not included in the MP test panel (n=14), 574 samples were included in the analysis. In total 212 (37%) samples were positive with at least one method, whereof 95 (17%) were positive in BC, 180 (31%) positive in MP and 54 (9%) positive in both methods. Fourteen samples were positive for more than one pathogen, 12 MP positive and 2 BC positive samples. Compared with BC, the following species specific sensitivities of MP were noted: *S. aureus*, 74% (14/19); *E. coli*, 54% (20/37); *S. pneumoniae* 46% (6/13). Table 1 shows the distribution of positive test results for all found pathogens.

Conclusions
In this study MP detected significantly more pathogens in blood than BC did, among patients with suspected sepsis. Notably is the much higher prevalence of *S. agalactiae* in MP (n=25) than in BC (n=1), where more than half of the positive MP results were confirmed with a species specific PCR method. The study indicates that *S. agalactiae* is under-diagnosed with BC.

Table 1.
Number of samples positive for MagicPlex Sepsis Real-time PCR (MP) and/or blood culture (BC) for different species. Number in brackets are samples confirmed by species specific inhouse PCR.

Pathogens	MP+/ BC+	MP+/ BC-	MP-/ BC+
Streptococcus pneumoniae	6 (4)	15 (1)	7 (0)
Streptococcus pyogenes	2	0	1
Streptococcus agalactiae	1 (1)	25 (13)	0
Streptococcus spp.	1	1	4
Staphylococcus aureus	14 (9)	35 (2)	5 (1)
Enterococcus faecalis	4	7	3
Escherichia coli[d]	20 (14)	30 (3)	17 (4)
Klebsiella pneumoniae	4	3	1
Klebsiella oxytoca	0	1	2
Enterobacter cloacae	3	5	0
Proteus mirabilis	0	0	1
Pseudomonas aeruginosa	0	4	3
Acinetobacter baumannii	0	10	0
Stenotrophomonas maltophilia	0	24	0
Serratia marcescens	0	1	0
Bacteroides fragilis	2	4	0
Total	57	165	44