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Multiplex PCR for rapid diagnosis of neonatal late onset sepsis

M. Van den Brand[1], F.A.M. Van den Dungen[2], A. Rubenjan[3], A.M. Van Furth[2], M.M. Van Weissenbruch[2], M.P. Bos[4], P.H.M. Savelkoul[1], R.P.H. Peters[5]

[1]Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, Netherlands ; [2]Pediatrics, VU University Medical Center, Amsterdam, Netherlands ; [3]Medical Mcirobiology and Infection Control, VU University Medical Center, Amsterdam, Netherlands ; [4]Research, Microbiome, Houten, Netherlands ; [5]Medical Microbiology, Maastricht University Medical Center, Maastricht, Netherlands

Objectives
Preterm infants are at high risk for acquiring nosocomial late onset sepsis (LOS) and accurate diagnosis and treatment is crucial due to its high morbidity and mortality. The diagnostic standard, blood culture, suffers from a long turnaround time and suboptimal sensitivity. Hence, a faster and accurate assay for detection of bacterial pathogens in blood is highly warranted. The use of broad-range PCR showed fair results but implementation is hampered by increased time needed for species identification and associated costs. Multiplex PCR has the benefit of direct species identification, but is rarely used for diagnosis of LOS. We clinically evaluated the diagnostic performance of a newly developed multiplex PCR that quantitatively detects the 8 most prevalent bacterial pathogens in LOS. This assay requires limited hands-on time and provides species-specific results within 4 hours.

Methods
We prospectively included 85 suspected episodes of LOS, occurring in 71 preterm neonates (gestational age <32 weeks) admitted to our NICU. A whole blood sample (0.2ml) for multiplex PCR was obtained together with blood culture prior to initiation of antibiotic therapy. Bacterial DNA was isolated using bacterial lysis buffer (Biocartis) and the EasyMag® system, and subjected to the multiplex PCR (panel: coagulase negative staphylococci, *Staphylococcus aureus, Enterococcus faecalis, Streptococcus agalactiae, Escherichia coli, Klebsiella spp., Pseudomonas aeruginosa and Serratia marcescens*). Blood culture and PCR were compared.

Results
Blood culture was positive in 57 and negative in 28 episodes, while PCR was positive in 51. For monomicrobial infections (n=78) PCR demonstrated a sensitivity of 80%, specificity 82%, PPV 89% and NPV of 70% compared to blood culture (Table 1). Seven episodes were polymicrobial, of which 4 were detected by PCR only. For example, the blood culture in one neonate with clinically necrotising enterocolitis revealed lactobacilli, while PCR detected DNA of *Klebiella spp*., *E. faecalis* and *S. agalactiae*.

Conclusions
We clinically evaluated a newly developed multiplex PCR that detects the 8 most common bacterial pathogens causing LOS in a cohort of preterm neonates with a high incidence of positive blood culture. PCR had a high positive predictive value of 89%, sensitivity of 80% and detected additional cases of possible LOS. To conclude, this study demonstrated that multiplex PCR is a useful additional diagnostic tool for rapid identification of LOS.

Diagnostic Performance of the Multiplex PCR Compared to Blood Culture for 78 Monomicrobial Infections

		Blood Culture +	Blood Culture -
Multiplex PCR	+	31 CoNS 6 *S. aureus* 2. *E. coli* 1. *S .agalactiae*	3 CoNS 1 *S. agalactiae* 1 *S. aureus*
	-	9 CoNS 1 *S. aureus*	23 negative

CoNS = coagulase negative staphylococci