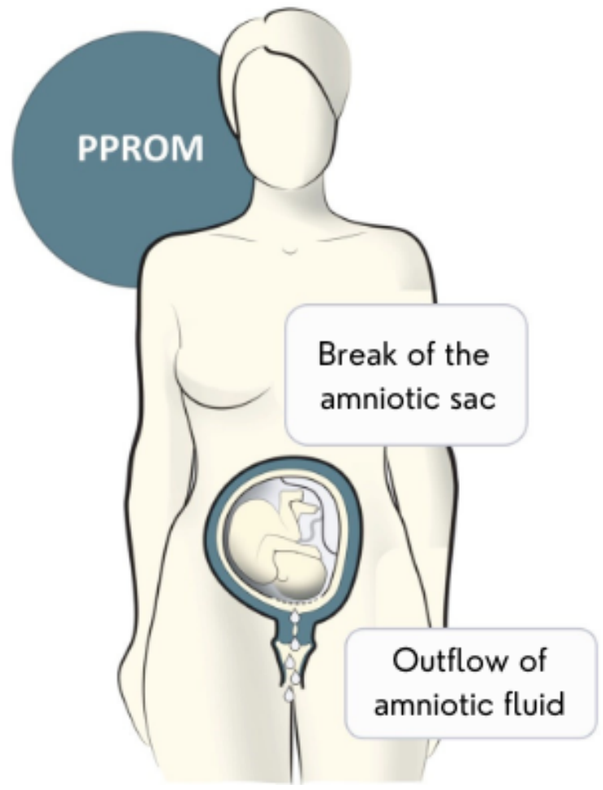
BACTERIAL DETECTION IN AMNIOTIC FLUID



TECHNOLOGY OWNER
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IPR STATUS
Know-how is fully owned by University Hospital Hradec Králové

STAGE OF DEVELOPMENT
Proof of Concept

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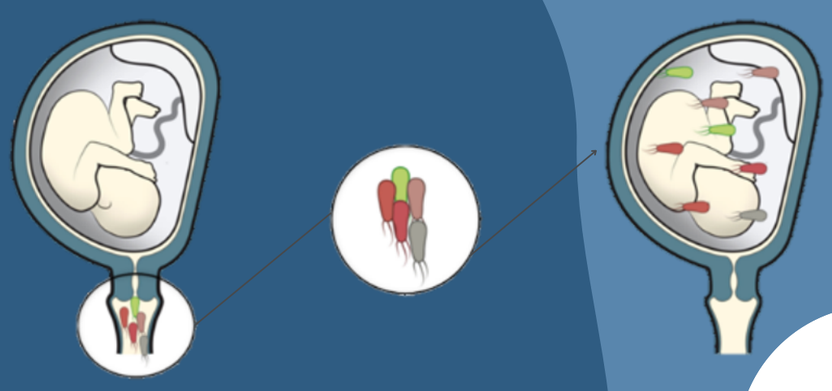
Background

Preterm Prelabour Rupture of Membranes (**PPROM**) is a **pregnancy complication**. In this condition, the sac (amniotic membrane) surrounding the fetus breaks (ruptures) before week 37 of pregnancy. Once the sac breaks, pregnant woman has increased risk for infection. PPROM complicates 3 – 4 % of all pregnancies and is up to **1/3 complicated by microbial invasion of the amniotic cavity (MIAC)** leading to infection in amniotic fluid (AF) and **development of intra-amniotic inflammation**. Although this complication is usually asymptomatic, it is a **major cause of preterm birth and neonatal morbidity and mortality worldwide**. Neonates from these pregnancies are at increased risk of developing neonatal sepsis, impaired psychomotor development and other sometimes lifelong health consequences. It is crucial to rapidly and accurately detect MIAC and identify pathogens in order to initiate targeted antibiotic treatment from the very beginning. Thus the fetus exposure to the infectious and inflammatory environment in amniotic cavity and the risk of its damage due to ongoing infection is minimized.

Description

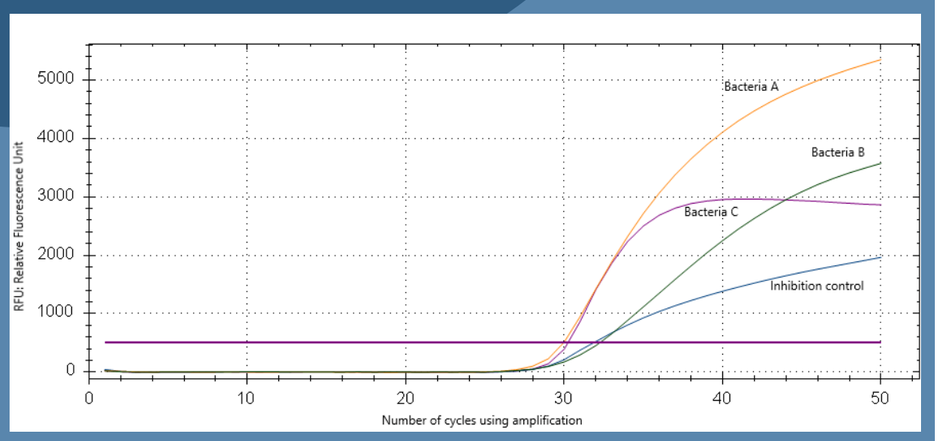
We have developed **multiplex Real Time - PCR** (Real - Time Polymerase Chain Reaction) **assay for simultaneous detection of the most common targeted microorganisms in AF** collected by amniocentesis. The method will **reduce the time required to diagnose specific pathogen and help clinicians make quick and accurate treatment decision**. The assay consists of 3 panels for DNA detection of 8 most common microorganisms. Each panel contains specific sets of primers and probes. Assay results are determined by PCR fluorescence signal. Panels were validated retrospectively on 20 samples of AF of PPROM patients. We have reached 90 % sensitivity so far. Further prospective validation study is currently underway.



Advantages

- **High sensitivity** of the method - panel of selected microorganisms in multiplex assay was defined on the basis of research in nearly 700 patients with PPROM and it covers 88 % of microorganisms responsible for MIAC in PPROM patients
- The multiplex approach **determines the selected microbes in several hours after sampling** in semi-quantitative way compare to the current approach (combination of specific multiplex Real Time - PCR for 3 microorganisms in combination with PCR assay targeting 16S rRNA regions followed by Sagner sequencing for the rest of targeted microbes and cultivation techniques)
- **Personalized approach** to the clinical management and therapeutic intervention of the patients with PPROM based on the assay results
- The technology **does not require specialized laboratory equipment** or specialized personnel and thus is suitable for molecular laboratory of perinatology centres equipped with standard real-time PCR cycler

Mixed amniotic fluid sample containing 3 bacteria (A, B, C) and inhibition control confirming the functionality of one of the 3 rt-PCR panels for MIAC detection



Currently, determining the MIAC in patients with PPROM is very time-consuming and technically demanding. It is based on combination of cultivation and molecular methods. Main disadvantage is that results are available in days, which is already clinically irrelevant for the initiation of targeted antibiotic treatment.

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