A new pre-amplification method to detect very low DNA copy numbers of (multi-)resistant pathogens in complex samples

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Abstract:

Detecting low copy-numbers of DNA and RNA in diagnostically relevant, complex samples, such as blood or pus, is a challenge. In clinical diagnostics, molecular assays gain an increasingly important role. The development of new specific and sensitive assays for the detection of pathogens is often hindered by the need to analyse complex clinical samples directly without previous cultivation. In addition to inhibitory and sample matrix effects, target molecules of RNA or DNA are usually present in very low concentrations. Up to now, most molecular tests for bloodstream infections start from a positive blood culture, where the causative agent and its genome will be enriched above the limit of detection for molecular tests. This requires several hours, thus delaying all subsequent analytical steps. Molecular assays that directly detect pathogens are limited in specificity, sensitivity and ability for multiplexing analyses of different targets.

Our newly developed pre-amplification method will help to close this gap. Starting from 10 mL venous blood followed by different filtration and lysis steps, a volume of 50 µl of purified DNA was further processed with a two-step pre-amplification method for many target sequences at once. In the first step, two chimeric primers per molecular marker with a target specific sequence part attached to a unique 20-mer oligo were used to pre-amplify and tag the target-DNA using a specific strand-displacement *Taq*-polymerase. In the second step, the tagged DNAs of a mixture of many targets are pre-amplified up to detectable levels. To verify the outcome, we used standard real-time PCR. With an initial treatment of 20 copies and 1µg of human DNA as background per reaction, the concentration of many different target DNAs was increased specifically to at least 1,000 copies/reaction.