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Isothermal Solid-Phase Amplification System for Detection of Yersinia pestis

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Abstract : DNA amplification is required for most molecular diagnostic applications but conventional PCR has disadvantages for field testing. Isothermal amplification techniques are being developed to respond to this problem. One of them is the Recombinase Polymerase Amplification (RPA) that operates at isothermal conditions without sacrificing specificity and sensitivity in easy-to-use formats. In this work RPA was used for the optical detection of solid-phase amplification of the potential biowarfare agent Yersinia pestis. Thiolated forward primers were immobilized on the surface of maleimide-activated microtitre plates for the quantitative detection of synthetic and genomic DNA, with elongation occurring only in the presence of the specific template DNA and solution phase reverse primers. Quantitative detection was achieved via the use of biotinylated reverse primers and post-amplification addition of streptavidin-HRP conjugate. The overall time of amplification and detection was less than 1 hour at a constant temperature of 37oC. Single-stranded and double-stranded DNA sequences were detected achieving detection limits of 4.04*10-13 M and 3.14*10-16 M, respectively. The system demonstrated high specificity with negligible responses to non-specific targets.

Keywords : recombinase polymerase amplification, Yersinia pestis, solid-phase detection, ELONA
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