Two-step DNA Isolation Followed by PCR in a Fully Automated System to Detect Septicemia Agents in Whole Blood

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Abstract

Objective: To develop a novel nucleic acid isolation and purification procedure on the Rheonix SeptiCARD® molecular diagnostic platform to achieve fully automated diagnosis of bloodstream infections within a three-hour time frame.

Methods

The design of the CARD device permits an untreated whole blood specimen to be directly applied in the Sample inlet reservoir (Figure 3) and under the control of software running the EncompassMDx™ Workstation, automatically perform all steps.

Relevance: The current “gold standard” method to detect bloodstream infections relies upon blood culture methods. The main disadvantages of this method are the time delays between availability of test results and the initiation of medical treatment as well as the low analytical sensitivity of the test. Since mortality rates in sepsis patients increase by approximately 8% for every hour of delay, alternatives need to be developed to overcome these shortcomings. PCR based molecular tests can potentially overcome many of these issues, however, the financial cost, personal training and the stringent lab controls required to prevent carryover and cross contamination remain serious roadblocks to widespread molecular testing in hospital labs. The Rheonix SeptiCARD is a low cost, high sensitivity and fully automated microfluidic device designed to conduct multiplexed microbial detection for sepsis diagnosis.

Relevance: For high sensitivity blood sample molecular testing, the purity and quality of the nucleic acid template is one of the most critical factors. We have developed a dual-stage purification method that incorporates both magnetic bead-based and silica column based nucleic acid isolation and purification schemes into a single Rheonix CARD® device for fully automated molecular analysis. Once an untreated whole blood sample is introduced, cell lysis, dual-stage DNA purification, multiple PCR and endpoint detection on a low density DNA array are automatically performed without any further user intervention. Validation: Whole blood was spiked with defined numbers of Candida albicans. E. coli and enterococcus and up to 1.5 ml samples were processed on the bench top and on the CARD. The total recovery and purity of isolated DNA was evaluated by comparing the 280/260 and 260/230 nm ratios, electrophoresis gels of the isolated genomic DNA and the PCR amplifications and the microarray results. Comparison between the single and dual-stage purification methods demonstrated higher PCR detection sensitivity for the dual-stage purified sample in every test case.

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Conclusions: The dual-stage nucleic acid purification scheme was developed and verified on the bench top. The concept was then incorporated into Rheonix SeptiCARD design and the device fabricated. When the SeptiCARD is placed into the Rheonix EncompassMDx™ workstation, which can simultaneously run six such CARD devices, up to 12 individual samples can be automatically analysed within a three-hour period. Carryover or cross contamination is also avoided by the closed nature of the Rheonix SeptiCARD device.