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 CARD® molecular diagnostic platform to achieve fully automated diagnosis of bloodstream infections within a three hour time frame.

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.

Methodology: 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, multiplex 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 260/280 nm and 260/230 nm ratios, electrophoresis gel of the isolated genomic DNA and the PCR amplicons 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.

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 analyzed within a three-hour period. Carryover or cross contamination is also avoided by the closed nature of the Rheonix SeptiCARD device.

Methods

Up to 1.5 ml of whole blood, spiked with *Candida albicans*, can be loaded into a CARD equipped to isolate DNA using either a single step (i.e., magnetic bead purification) or an orthogonal two step DNA isolation (magnetic bead adsorption, followed by silica membrane isolation). The recovery and quality of DNA was determined by spectrophotometric analysis and gel electrophoresis. In addition, PCR amplification of the isolated DNA by these two approaches was compared.

In order to accommodate the volume of whole blood that was automatically processed through the CARD, adaptors were fabricated that would permit a standard Luer-Lok syringe to be locked into place (Figure 1). The CARD was designed to perform the orthogonal DNA isolation when placed into the EncompassMDx™ workstation as shown (Figure 2).

Figure 1: CARD equipped to process untreated whole blood

Figure 2: EncompassMDx™ Workstation

Comparison of Magnetic Bead-Based and Orthogonal Dual-stage DNA Isolation Methods

Equal 1.5 ml aliquots of whole blood, spiked with *Candida albicans* cells, were introduced into a Rheonix SeptiCARD device equipped to isolate DNA using either magnetic bead purification or a magnetic bead isolation followed by a silica membrane purification. Once the DNA isolation process was completed, the quality and quantity of DNA isolated was compared by spectrophotometric analysis and gel electrophoresis. Additionally, the ability to PCR amplify the isolated DNA was compared. In general the two different processes were performed as follows:

Magnetic Bead DNA Isolation

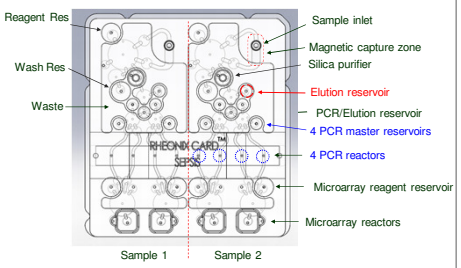
1. Sample applied
2. Cells lysed
3. Magnetic Bead isolation of DNA
4. PCR Amplification

Orthogonal DNA Isolation

1. Sample applied
2. Cells lysed
3. Magnetic Bead isolation of DNA
4. Silica Membrane DNA purification
5. PCR Amplification

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.

Figure 3: Layout of CARD designed to perform orthogonal DNA isolation



Results & Conclusions

The design of the CARD device permits an untreated whole blood specimen to be applied in the Sample inlet reservoir (Figure 3) and under the control of software running the EncompassMDx™ workstation, automatically perform all steps. Although apparent total recovery of DNA (Figure 4) is lower with the two-step versus single step purification, the absorbance ratios at both 280/260 (Figure 5) and 260/230 (Figure 6) are considerably improved using the orthogonal two-step process. In addition, the quality of the isolated genomic DNA as observed by gel electrophoresis (Figure) 7 is markedly improved after the two step process.

500 µl of whole blood, spiked with either 50 or 500 *C. albicans* cells, was added to the CARD and run through the entire orthogonal DNA isolation and PCR amplification. As compared to a parallel run using only magnetic bead capture of nucleic acids, the orthogonal DNA isolation approach yields DNA that is more effectively amplified using PCR. Ongoing experiments using other microorganisms common in septicemia are yielding similar improved PCR results following orthogonal DNA isolation.

Figure 4: Recovery of DNA

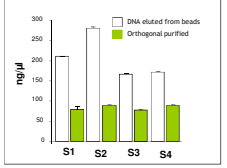


Figure 5: 280/260 Absorbance Ratios

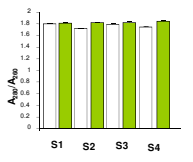


Figure 6: 260/230 Absorbance Ratios

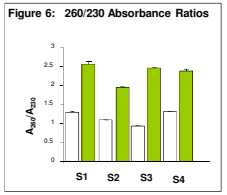


Figure 7: Isolated Genomic DNA

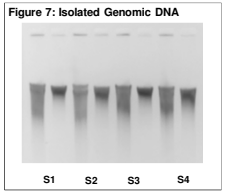


Figure 8: PCR Detection of *C. Albicans* in whole blood

The use of an orthogonal DNA isolation procedure that includes adsorption to magnetic beads followed by silica membrane isolation yields DNA of superior quality for subsequent PCR amplification. When the system currently being optimized was tested using whole blood spiked with *C. albicans*, the on-CARD purification and PCR amplification of target sequences was easily achieved.

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