FILTER- CAPTURE DNA ISOLATION: A FILTER STERILIZATION AND NUCLEIC ACID PREPARATION METHOD FOR THE DETECTION OF BURKHOLDERIA PSEUDOMALLEI IN URINE

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Abstract (Revised)
The FCDI (filter capture DNA isolation) method is a nucleic acid preparation method for the rapid and sensitive detection of Burkholderia pseudomallei (Bp) in human urine samples using quantitative polymerase chain reaction (qPCR). In the U.S. laboratory work with cultures of Bp must be performed in BSL2 facilities, however Bp DNA isolation processes are often transferred to BSL3 for qPCR analysis after confirmation of lysate sterility. The objectives of the current study were to evaluate: (1) the sterilization performance, (2) optimal operational parameters, and (3) qPCR detection range and sensitivity of FCDI. For the current study, we applied human urine with the non-virulent Bp lab strain (Bp82) to spiked human urine for PCR analysis. The FCDI method was performed in less than one hour. No growth was observed from 65 urine samples after 5 days incubation, and PCR yielded a detection range and sensitivity of 4.1 X 10^4 to 4.1 X 10^5 CFU/ml. PCR analysis was also performed in cells spiked with Bp82 and Bp DNA detection was observed in FCDI lysates cultured for 5 days on SBA. The FCDI method demonstrates a robust performance in the detection of Bp in human urine with sensitivity (≤ 4.0 x 10^5 CFU/ml) at clinically relevant concentrations (Burkhartman 2007; Lederer et al, 2017). A standardized Bp82 spiked urine sample can be processed for detection of Bp in a BSL2 laboratory and then swiftly moved to a biosafety level 3 laboratory for PCR testing with results the same day.

Materials & Methods

Bacterial strains: Several Bp strains were used in the study. The predominant strain used was Bp82 (ATCC 25922), which was isolated from a Thai melioidosis patient. Bp strains were used to spike human urine samples. All Bp strains were cultured on plates of tryptic soy agar (TSA) and smears for morphological observations.

Growth & bacterial quantification: Bacterial suspensions of Bp were prepared in sterile saline and were used to inoculate urine for growth. A standard curve was prepared using serial dilutions of cells. Growth of Bp82 was determined after 5 days of incubation on TSA at 35°C in the presence of 5% CO2.

PCR detection range and sensitivity: The detection range of the PCR method was determined using serial dilutions of Bp strains. Sensitivity was determined using a standard curve of Bp82 DNA, with results the same day.

Results

**FCDI Method Steps:**

1. Cells washed with buffer and centrifuged at 350 g for 5 min.
2. Residual buffer removed by another centrifugation.
3. Cells on filter are lysed with 65 µl of 0.05 N NaOH (in 250 mM NaCl, 1 mM EDTA) and incubated at 35°C for 15 min.
4. Bp DNA lysates were collected by centrifugation, pH ashed with buffer and resuspended in saline before and after supernatants were analyzed for viable Bp82.
5. Bp lysate collected by centrifugation, pH neutralized and ready for PCR analysis.

**Figure 2:** Assessment of Bp82 from spiked urine (~10^6 CFU/ml) before and after the FCDI alkaline treatment of 15 min at 37°C. Bp DNA detection from FCDI lysates were collected by centrifugation, pH neutralized and ready for PCR analysis.

**Figure 3:** Range of Bp Detection by TTSI real-time PCR

<table>
<thead>
<tr>
<th>Range of Bp Detection (DNA)</th>
<th>Bp82 Spiked into Human Urine (CFU/ml)</th>
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<tbody>
<tr>
<td>4.1 X 10^4</td>
<td>23.7</td>
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<tr>
<td>4.1 X 10^5</td>
<td>30.8</td>
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<tr>
<td>4.1 X 10^6</td>
<td>37.2</td>
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**Table 1:** Sensitivity of Bp DNA by real-time PCR using FCDI lysates. Bp DNA was detected in spiked urine with Bp82 (250 PCR cycles) per concentration tandem.

Conclusions

- **Viable Bp could not be recovered from Bp lysates prepared from human urine spiked to concentrations of ~10^6 CFU/ml.**
- Prior to the alkaline step of FCDI, Bp cells captured on the 0.1µm filter are visible. Treatment of 0.1µm filters with 0.05% sodium hydroxide rendered lysates sterile.
- Up to 20 urine samples can be safely processed by the FCDI method in one hour. By real-time PCR, FCDI lysates yield a detection range and sensitivity of 4.1 X 10^4 to 4.1 X 10^5 CFU/ml for Bp82.

Discussion

From less than 1 ml of urine sample, lysates that are free of viable organisms and immediately available for PCR can be rapidly generated using the FCDI method. The FCDI method reduces laboratory hands-on time and utilizes shelf stable reagents for extraction. The FCDI method demonstrates a robust performance in the detection of Bp in human urine with sensitivity (≤ 4.0 x 10^5 CFU/ml) at clinically relevant concentrations (Burkhartman 2007; Lederer et al, 2017). A standardized Bp82 spiked urine sample can be processed for detection of Bp in a BSL2 laboratory and then swiftly moved to a biosafety level 3 laboratory for PCR testing with results the same day.

References and Acknowledgements

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