Evaluation of the JBAIDS Scrub Typhus and Rickettsia Detection Kits

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INTRODUCTION

The Joint Biological Entity Identification and Diagnostic System (PBDS) is the Department of Defense’s (DoD) Program of Record for the detection and diagnostic testing of biological warfare agents and infectious diseases of operational concern. Idaho Technology, Inc. (ITI), the developer of the PBDS platform, has developed real-time PCR assays that are intended to be in-vitro diagnostic (IVD) kits for the detection of scrub typhus and Rickettsia infections. The PBDS system is an FDA cleared device for in vitro diagnostic (IVD) testing and consists of the following components:

- thermal cycler and real-time reader
- Room temperature reagents that are hose-in and specific for the target organism
- Flagged/flagless tested with user friendly software
- Specific sample purification kits and protocols

The PBDS Scrub Typhus Detection Kit and PBDS Rickettsia Detection Kit were evaluated to determine if the assay would be submitted to the FDA for review for 510k approval. The assay is in process to be included in the FDA cleared IVD assays for the PBDS platform that now include detection kits for anthrax, plague, tularemia, Q-fever, and various influenza (A/H176N). The PBDS Scrub Typhus Detection Kit is designed to detect Orientia tsutsugamushi, the causative agent of scrub typhus, and the PBDS Rickettsia Detection Kit is designed to detect all Rickettsia species, which are infectious agents responsible for a variety of spotted fever and typhus like illnesses.

RESULTS

The Rickettsia assay primers and probes were designed to target a 120 bp region of a conserved sequence of 21 Rickettsia species. The assay was tested using genomic DNA from 16 different Rickettsia species and strains as well as two non-Rickettsia species. The assay successfully detected all Rickettsia organisms and the strain-Rickettsia (Table 1). Sensitivity was determined for the sequenced strains, R. bellii and R. canadensis were low. However, these species are not thought to be clinically relevant.

The JBAIDS Scrub Typhus assay primers and probes were designed to target a 150 bp region of the ompA target sequence of R. conorii, R. rickettsii and R. prowazekii. In previous studies, the primer pair successfully detected 19 different strains of O. tsutsugamushi, but not O. maynei.

Though easily detectable, O. maynei and Rickettsia infections are very difficult to diagnose because symptoms of early disease are identical to other diseases of similar epidemiology. Current diagnostic methods are serology-based and compare acute and convalescent phase antibody titers to the pathogens of interest. Current Rickettsia detection kits are available for scrub typhus (Orientia tsutsugamushi) and murine typhus (Rickettsia typhi). Sensitivity of the JBAIDS Rickettsia Detection Kit was evaluated to determine if the assay would be submitted to the FDA for review for 510k approval. The assay is in process to be included in the FDA cleared IVD assays for the PBDS platform that now include detection kits for anthrax, plague, tularemia, Q-fever, and various influenza (A/H176N). The PBDS Scrub Typhus Detection Kit is designed to detect Orientia tsutsugamushi, the causative agent of scrub typhus, and the PBDS Rickettsia Detection Kit is designed to detect all Rickettsia species, which are infectious agents responsible for a variety of spotted fever and typhus like illnesses.

Determination of naLoD using synthetic DNA

The nucleic acid limit of detection (naLoD) was determined for both the target and IC species by testing serial dilutions of a synthetic single-stranded DNA chip-compatible template. The naLoD was found to be 25 copies for both target assays.

Clinical Specimens

Residual, de-identified, frozen serum samples were tested for Rocky Mountain spotted fever (RMSF; R. rickettsii) using the JBAIDS Rickettsia Detection Kit at the North Carolina State Lab for Public Health in Raleigh, North Carolina. Specimens were selected from the Special Serology laboratory testing database and chosen for testing if they met the following criteria:

- An acute and convalescent specimen were available
- Seroconversion on a 1:4 or 8:1 dilution of IgG to R. rickettsii was observed
- At least 800 μL specimen volume

Table 2: Confirmation of naLoD

<table>
<thead>
<tr>
<th>Target DNA</th>
<th>IC DNA</th>
<th>Assay results</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. prowazekii, R. conorii, R. typhi</td>
<td>O. tsutsugamushi, O. maynei</td>
<td>5.15 (SD 1.12)</td>
</tr>
<tr>
<td>1.03</td>
<td>1.17</td>
<td>32.99</td>
</tr>
<tr>
<td>1.85</td>
<td>0.24</td>
<td></td>
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</tbody>
</table>

Seven scrub typhus specimens were tested, 4 of which gave positive results (4/7: 57.1%). Of the 2 murine typhus specimens tested, 4 were ruled positive and 2 were negative (one capillary positive, one negative). Sensitivity was 44.4% (4/9). Equine serum samples were not collected for testing volume. The multiplex inhibition control was performed as expected, indicating the negative results were not due to PCR inhibition.

Sample Purification

The JBAIDS assay is a simple-to-use Wizard-based interface. The instrument automatically calculates the threshold cycle (Ct) values for each sample and makes a Positive, Negative, Uncertain, or Invalid call.

CONCLUSION

The results presented here offer a preliminary assessment of the JBAIDS Scrub Typhus and Rickettsia Detection Kits for diagnostic, serological and clinical specimens. The assay are able to detect at least 25 copies of synthetic DNA chip-compatible template and 25-50 genomic equivalents of purified bacterial DNA per reaction. Sensitivity of the JBAIDS Rickettsia Detection Kit for RMSF is ≥ 1200 copies per μL for murine typhus and ≥ 800 copies per μL for scrub typhus. The JBAIDS Scrub Typhus Detection Kit has a sensitivity of ≥ 100 copies per μL in buffy coat. While a negative PCR result is not definitive for the absence of Orientia or Rickettsia infection, a positive test result is an excellent indication of infection, particularly early in the course of the disease. The PBDS platform can be extended to include other Rickettsia species, including O. tsutsugamushi, scrub typhus, and Rickettsia species, and murine typhus. The JBAIDS scrub typhus and murine typhus kits were also evaluated using the JBAIDS Rickettsia Detection Kit assay. Data, including serology results, are summarized below.

Clinical Specimens – Scrub Typhus and Murine Typhus (SMRU)

Residual buffy coat extracts from acute (14) and patients with serologically confirmed murine and scrub typhus were obtained from the Shikoku Medical Research Unit in Mea Sorl, Thailand. Specimens were collected as follows: 5 mL of EDTA blood was centrifuged to separate plasma, red blood cells, and buffy coat. Ten hundred microliters (250 μL) of buffy coat was removed and extracted with the QIAamp DNA Blood nucleic acid kit. Extract was stored at -80°C and shipped to ITI on dry ice.

Scrub typhus samples were analyzed using the JBAIDS Scrub Typhus Detection Kit assay. Murine typhus samples were analyzed using the JBAIDS Rickettsia Detection Kit assay. Data, including serology results, are summarized below.