INTRODUCTION
The JBAIDS Typhus Detection Kit: a Real-time PCR-based assay for the detection of Rickettsia and Orientia tsutsugamushi infestations

Kevin M. Bourzac,1* Peta Koike,2 Alix Anderson,1 Cody Oswald,1 Matthew Jones,1 Ju Jiang,1 HongGer,1 Allen L. Richards,1 James W. Karasick,2 Jennifer C. McLaunghlin, and Beth Lingerfelt1

1Math Tech, Inc., 2Large Lake City, UT, Naval Medical Research Center, Silver Spring, MD, Chemical Biological Medical Systems, JPA-Bioassurance (provisional), Frederick, MD

THE JBAIDS Typhus Detection Kit consists of two multiplexed real-time PCR assays: one for the specific detection of Orientia tsutsugamushi and another for the detection of Rickettsia spp. Both designs consist of a synthetic DNA construct containing the primers and sequence targets of the assay and a heterologous intervening sequence to which is linked probe triplets. The target assay is read in Channel 2 (705 nm); the IC is read in Channel 3 (1310 nm).

Rickettsial Infection & Host Response

Nucleic acids are isolated from whole blood and serum specimens for analysis with the JBAIDS Typhus Detection Kit. PCR primers and probes are designed against a target that was selected from a conserved sequence of 21 Rickettsiae species. The assay was tested using genomic DNA from 14 different Rickettsiae species and strains as well as two non-Rickettsiae species. The pan-Rickettsiae assay was successfully detected at Rickettsiae organisms and non-Rickettsiae (Table 1). Sensitivities for the ancestral species, R. rickettsii and R. conorii, were low. However, these species are not thought to be clinically relevant.

RESULTS

The pan-Rickettsiae/Assay Detects Multiple Rickettsiae species

An easily testable, Rickettsia and O. tsutsugamushi infections are very difficult to diagnose because symptoms of early disease are identical to other diseases of similar epidemiology. Current diagnosis methods are based on serosentimental methods which compare a single and convalescent antibody titers. As such, they require sufficient time for seroconversion (1-2 weeks); thus they are not always reliable for the diagnosis of early-stage disease. A delayed diagnosis (and treatment) leads to a higher mortality rate. In contrast, the real-time PCR assay in the JBAIDS Typhus Detection Kit can be performed during optimal disease diagnosis during the acute phase of disease leading to earlier treatment and recovery.

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The JBAIDS system is an FDA-cleared device for in vitro diagnostic testing and consists of the following components:

• JBAIDS instrument with thermocycler and real-time fluorimeter
• Room temperature-stable reagents that are freeze-dried and specific for the target organism
• Ruggerized laptop loaded with specific, user-friendly software
• Specific single-patient kits and protocols

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The Assays Remain Sensitive in the Presence of Human Genomic DNA

The IT-1-2-3 Platinum Path extraction kit purifies all nucleic acids found in a sample, not just those of microbial origin. Therefore, it is important to ensure that the assays do not lose sensitivity in the presence of human genomic DNA (hDNA) that may be purified along with any bacterial DNA. To test for hDNA interference, reactions were spiked with synthetic template and tested with and without 1.5 µg of hDNA per reaction. It was found that the presence of hDNA does not alter the ability of either assay to detect synthetic target spiked at naLoD (Table 3).

Inhibition Control Performance in Whole Blood Samples

To test the reproducibility of the IC using the intended sample matrix, equilibrated whole blood samples from 21 healthy volunteers were purified using the IT-1-2-3 Platinum Path DNA Extraction Kit and tested using both assays. The IC gave a positive result for all replicates of the IC spiked whole blood samples. In the case of the pan-Rickettsiae assay, all replicates were positive except for one IC. Analysis of the data showed that an amplification curve was observed, however the software made a negative call due to low fluorescence (not shown).

Table 4: IC performance using purified whole blood samples

<table>
<thead>
<tr>
<th>Species</th>
<th>Assay</th>
<th>Spike Level</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. prowazekii</td>
<td>IC</td>
<td>5,000 org/mL</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>O. tsutsugamushi</td>
<td>whole-blood</td>
<td>5,000 org/mL</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Detection of Rickettsia and Orientia in Whole Blood Samples

Next, we tested the assay performance on whole blood samples. Inoculated R. prowazekii and O. tsutsugamushi isolates were spiked into whole blood at 1,000, 2,000, and 5,000 genomic equivalents. Results are presented in Table 5. The pan-Rickettsiae assay was capable of detecting spiked R. prowazekii at all spike levels. Results for O. tsutsugamushi show the assay is not as sensitive; this is consistent with naLoD data from genomic DNA (see Table 5).

Table 5: naLoD of spiked organism in whole blood

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC</th>
<th>Spike Level</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>whole-blood</td>
<td>IC</td>
<td>5,000 org/mL</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

CONCLUSION

Several studies are planned to continue the development of the JBAIDS Typhus Detection Kit. These include:

• Development of Channel 2 chemistry to increase robustness of IC assay
• Clinical Validation
• A multi-site prospective study involving collection and testing of whole blood and buffy coat samples from patients with suspected tick-borne or ’typhus-like’ illnesses
• Trials to support a 510(k) application to the FDA for approval of the JBAIDS Typhus Detection Kit as an in vitro diagnostic assay
• Analytical evaluation studies
• Clinical trial at multiple sites worldwide, anticipated May-October 2011

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This work is performed under DoD contract No: DASG60-03-0894