



Evaluation of the JBAIDS Scrub Typhus and Rickettsia Detection Kits

Kevin M. Bourzac^{1*}, Kim A. Zullo², Wanitda Watthanaworawit³, Shermalyn Greene², Paul Turner³, Ju Jiang⁴, Allen L. Richards⁴, James W. Karaszkiwicz⁵, Jennifer C. Dabisch⁵, and Beth Lingenfelter¹
¹Idaho Technology, Inc., Salt Lake City, UT, ²North Carolina State Lab of Public Health, Raleigh, NC, ³Shoklo Malaria Research Unit, Mae Sot, Thailand, ⁴Naval Medical Research Center, Silver Spring, MD, ⁵JBAIDS Program Office, JPM Biosurveillance (provisional), Frederick, MD

INTRODUCTION

The Joint Biological Agent Identification and Diagnostic System (JBAIDS) 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 JBAIDS platform, has developed real-time PCR assays that are intended to become *in vitro* diagnostic (IVD) kits for the detection of scrub typhus and Rickettsia infections.

The JBAIDS system is an FDA-cleared device for *in vitro* diagnostic (IVD) 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
- Ruggedized laptop loaded with specific, user-friendly software
- Specific sample purification kits and protocols.



RESULTS

The Rickettsia assay detects multiple Rickettsia species

The Rickettsia assay primers and probes were designed against a target that was selected from a consensus sequence of 21 Rickettsia species. The assay was tested using genomic DNA from 14 different Rickettsia species and strains as well as two non-Rickettsia species. The Rickettsia assay successfully detected all Rickettsia organisms and no non-Rickettsia (Table 1). Sensitivities for the ancestral species, R. bellii and R. canadensis, were low. However, these species are not thought to be clinically relevant.

Table 1: Rickettsia assay inclusivity and exclusivity

Strain	Detected
R. amblyommii 85-1084	+++
R. bellii G2D	+
R. bellii OT-2006	+
R. canada* MCK-29(29)	+
R. canadensis CA410	+
R. conorii VR597 ITT	+++
R. felis Cal2	+++
R. montanensis OSU 85-930	+++
R. prowazekii Breinl	+++
R. prowazekii Madrid E	+++
R. rickettsii VR891 R	+++
R. sibirica 246 CWPP	+++
R. slovaca Arm25	+++
R. typhi Wilmington	+++
O. tsutsugamushi Kato	-
C. burnetti NMQ	-

*R. canada has been renamed R. canadensis

The O. tsutsugamushi assay primers and probes were designed to a target using the consensus sequence of Karp, Kato, Gilliam and Boryong strains. In previous tests, this primer pair successfully detected 19 different strains of O. tsutsugamushi but no non-Orientia.

Strain-specific naLoD using bacterial genomic DNA

R. prowazekii, R. conorii, R. rickettsii, R. typhi and O. tsutsugamushi genomic DNA (calculated in genomic equivalents, GE) were serially diluted and spiked into the appropriate reconstituted freeze-dried reagents. The naLoD for each strain was determined and are listed in Table 3.

Table 3: naLoD

Species	Strain	JBAIDS Assay	naLoD (GE)
R. prowazekii	Brein 1	Rickettsia	25
R. conorii	VR597 ITT	Rickettsia	50
R. rickettsii	Sheila Smith	Rickettsia	25
R. typhi	Wilmington	Rickettsia	50
O. tsutsugamushi	Karp	Scrub Typhus	150

Clinical Specimens - RMSF

Residual, de-identified, frozen serum specimens 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 was available
- Serconversion or a $\geq 4x$ rise in IgG to R. rickettsii was observed
- At least 800 μ L specimen volume

Twelve (12) patients met this criteria. Nucleic acid was extracted using the IT 1-2-3 Platinum Path nucleic acid purification kit.

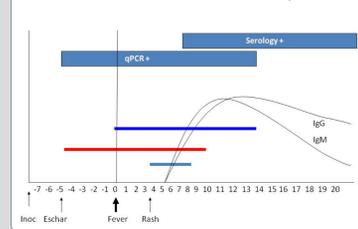
Table 4: Serum specimens tested at NCSLPH

Patient	Acute		Convalescent		JBAIDS
	Days post onset	Titer	Days post onset	Titer	
1	5	1:64	68	$\geq 1:256$	neg
2	4	1:64	18	$\geq 1:2048$	neg
3	2	<1:64	18	$\geq 1:256$	neg
4	4	1:64	18	$\geq 1:256$	neg
5	2	1:64	16	$\geq 1:256$	neg
6	4	1:128	18	1:512	neg
7	4	<1:64	18	$\geq 1:256$	pos
8	4	1:64	18	$\geq 1:256$	pos
9	unknown	1:64	unknown	$\geq 1:256$	neg
10	1	1:128	15	1:1024	neg
11	5	1:128	22	$\geq 1:2048$	pos
12	4	<1:64	18	$\geq 1:256$	neg

Twelve (12) acute specimens were tested using the JBAIDS Rickettsia Detection Kit, 3 of which were positive (3/12; 25%). Convalescent specimens were tested as well; all were negative. The internal IC was positive in all reactions that were negative for the target, indicating that negative results were not due to PCR inhibition.

All positive specimens were subject to additional analysis consisting of sequencing of 3 gene targets: 17kDa antigen gene, OmpA and OmpB. Results obtained from Patient 11 were confirmed to be R. rickettsii. No further data was obtained from the other 2 patients due to very low analyte levels that were insufficient for analysis.

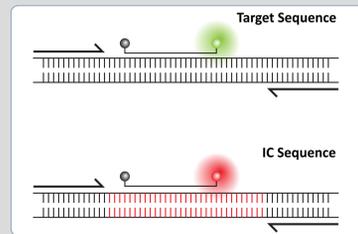
Rickettsial Infection & Host Response



The JBAIDS Scrub Typhus Detection Kit and 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 line to be included in the FDA-cleared IVD assays for the JBAIDS platform that now include detection kits for Anthrax, Plague, Tularemia, Q-Fever and Avian Influenza (A/H5 lineage).

The JBAIDS Scrub Typhus Detection Kit is designed to detect Orientia tsutsugamushi, causative agent of scrub typhus, and the JBAIDS Rickettsia Detection Kit is designed to detect all Rickettsia species, which are infectious agents responsible for a variety of spotted fever and typhus illnesses.

Though easily treatable, O. tsutsugamushi and Rickettsia infections are very difficult to diagnose because symptoms of early disease are identical to other diseases of similar epidemiology. Current diagnosis methods are serology-based and compare acute and convalescent phase antibody titers to the pathogens of interest. As such, they require sufficient time for seroconversion; thus they are not always reliable to detect disease in the early stages. A delayed diagnosis (and treatment) leads to a higher mortality rate. In contrast, the real-time PCR assays in the JBAIDS detection kits are intended to provide rapid diagnosis during the acute phase of disease.



The JBAIDS Scrub Typhus and Rickettsia Detection Kits contain freeze-dried real-time PCR assays. Both assays are multiplexed with an internal inhibition control (IC) that consists of a synthetic DNA construct containing the primer binding sequences of the target assay and a heterologous intervening sequence to which a novel probe binds. The target assay is read in Channel 1 (530 nm); the IC is read in Channel 3 (705 nm).

Both kits were evaluated using synthetic and purified nucleic acids as well as specimens from patients with serologically confirmed Rocky Mountain spotted fever (RMSF; R. rickettsii), murine typhus (R. typhi), and scrub typhus (O. tsutsugamushi).

Determination naLoD using synthetic DNA

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

Table 2: Confirmation of naLoD

	Scrub Typhus		Rickettsia	
	Cp (SD)	F _{max} (SD)	Cp (SD)	F _{max} (SD)
Target	33.35 (0.31)	2.94 (0.91)	33.32 (0.46)	6.15 (1.38)
IC	33.00 (0.23)	1.17 (0.13)	32.19 (0.24)	1.55 (0.28)

Clinical Specimens – Scrub Typhus and Murine Typhus (SMRU)

Residual buffy coat extracts from acutely ill patients with serologically confirmed murine and scrub typhus were obtained from the Shoklo Malaria Research Unit in Mae Sot, Thailand. Specimens were collected as follows: 6 mL of EDTA blood was centrifuged to separate plasma, red blood cells, and buffy coat. Two hundred microliters (200 μ L) of buffy coat was removed and extracted with the Qiagen 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.

Table 5: Scrub Typhus Buffy Coat Extracts, Serology and JBAIDS Results

Patient	Acute		Convalescent		JBAIDS
	Days post onset	Titer	Days post onset	Titer	
1	5	1:800	14	$\geq 1:25600$	neg
2	3	1:400	13	1:6400	pos
3	5	1:400	15	$\geq 1:25600$	pos
4	6	1:200	17	$\geq 1:25600$	pos
5	1	$\geq 1:100$	11	1:3200	neg
6	2	1:200	16	1:1600	neg
7	5	1:1600	12	$\geq 1:25600$	pos

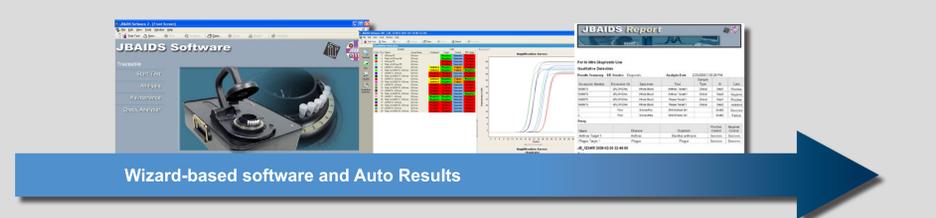
Table 6: Murine Typhus Buffy Coat Extracts, Serology and JBAIDS Results

Patient	Acute		Convalescent		JBAIDS
	Days post onset	Titer	Days post onset	Titer	
8	6	1:400	16	1:3200	equiv
9	4	1:400	14	$\geq 1:25600$	pos
10	4	1:200	13	1:3200	pos
11	6	1:400	16	1:12800	pos
12	6	1:200	17	1:6400	pos
13	3	1:200	13	1:12800	neg
14	3	1:400	15	$\geq 1:25600$	neg
15	3	1:200	13	1:6400	equiv
16	3	1:100	13	1:1600	neg

Seven scrub typhus specimens were tested, 4 of which gave positive results (4/7; 57.1%). Of the 9 murine typhus specimens tested, 4 were called positive and 2 gave equivocal results (one capillary positive, one negative). Sensitivity was 44.4% (4/9). Equivocal calls were not retested due to limited sample volume. The multiplexed inhibition control performed as expected, indicating the negative results were not due to PCR inhibition.

Nucleic acids are isolated from specimens for analysis with the JBAIDS assays by purification using the IT 1-2-3 Platinum Path sample purification kit. In this simple-to-use kit, bead-beaten specimens are incubated with magnetic beads which bind nucleic acids. The beads are then moved through a series of washes using a magnetic tool and finally eluted in buffer. Purified sample is combined with reconstitution buffer to rehydrate freeze-dried PCR reagents, which are then loaded into glass capillaries and run on the JBAIDS instrument.

The JBAIDS instrument is controlled by a simple-to-use Wizard-based interface. The instrument automatically calculates crossing point (Cp) values for each sample and makes a Positive, Negative, Uncertain, or Inhibited call.



CONCLUSION

The results presented here offer a preliminary assessment of the JBAIDS Scrub Typhus and Rickettsia Detection Kits for synthetic template, genomic DNA, and clinical specimens. The assays are able to detect at least 25 copies of synthetic template and 25-50 genomic equivalents of purified bacterial DNA per reaction. Sensitivity of the JBAIDS Rickettsia Detection Kit for RMSF is 25% in serum specimens and 44.4% for murine typhus in buffy coats. The JBAIDS Scrub Typhus Detection Kit has a sensitivity of 57.1% in buffy coats. 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 when serology is negative. A larger, prospective evaluation is currently underway in Thailand that will assess serum, whole blood, and buffy coat specimens from patients with non-malarial, undifferentiated fevers. The results of this study will be used to determine the best sample type to proceed with further testing (analytical evaluations and a full clinical trial) that will be undertaken in order to support an application for FDA clearance of these kits as *in vitro* diagnostics.

This work is performed under DoD contract No: DASG60-03-0094

See all of ITI's scientific posters by scanning the QR code to access the Scientific Poster Page



CONTACT INFORMATION
 Kevin Bourzac, Ph.D.
 Idaho Technology Inc.
 kevin_bourzac@idahotech.com

Presented at 6th International Meeting on Rickettsia and Rickettsial Diseases, June 2011

