

# Joint Biological Agent Identification and Diagnostic System (JBAIDS): Assay Validation

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## Summary

A real-time Polymerase Chain Reaction (PCR) system coupled with sample extraction kits, and room temperature stable freeze-dried reagents shows equivalent performance levels at initial testing and 6 month storage. Six matrices of clinical and environmental relevance were tested with 30 assays designed to detect 14 bio-threat organisms.

## Abstract

The goal of the Joint Biological Agent Identification and Diagnostic System (JBAIDS) program was to develop a portable system capable of simultaneous identification of multiple bio-threat agents and other pathogens of operational significance. The JBAIDS platform consists of a real-time PCR-based instrument, along with a laptop computer with software that generates automated detection calls. This platform is used in conjunction with sample extraction kits and room temperature stable freeze-dried (FD) reagents. Sixteen assays have been developed to detect diseases of bacterial or viral origin: Anthrax, Smallpox, Plague, Tularemia, Brucellosis, Q fever, Glanders, Typhus, viral Encephalitis and hemorrhagic fever. Two key JBAIDS Validation Tests (JVTs) were conducted per pathogen to assess the sensitivity of the overall system and stability of FD reagents stored at 26°C-30°C. These JVTs involved processing of simulated samples (6 matrices) spiked with multiple concentrations of gamma-irradiated organism. Nearest neighbor (NN) tests were also conducted to assess the assay specificity. All of the freeze-dried assays tested exhibited sensitivities that met or exceeded 85% success (at 85% or 95% confidence) at the following pathogen levels: 10<sup>3</sup> cfu or 10<sup>4</sup> pfu/ml whole blood or PBS, 10<sup>3</sup> cfu or 10<sup>4</sup> pfu/20 ml culture media, 10<sup>3</sup> cfu or 10<sup>4</sup> pfu/mg powder, and 10<sup>3</sup> cfu or 10<sup>4</sup> pfu/swab (nasal or surface). Samples spiked with 10-fold lower levels of gamma-irradiated organism revealed that the system could detect most of these samples as well. All assays passed the NN tests. The JVTs showed that the FD reagents maintain sensitivity and specificity performance levels after 6-months of storage at 26°C-30°C. Ongoing stability studies support that these FD reagents will be stable out to a year.

## Background

The September 11 terrorist attack on the World Trade Center and the anthrax postal attacks of 2001 signaled the need for a compact diagnostic tool with capabilities to quickly identify biothreat agents. The Joint Biological Agent Identification and Diagnostic System (JBAIDS) represents the Department of Defense's (DoD) first effort to develop and field a common platform that will identify biological warfare agents. The combination of a commercial-off-the-shelf platform and reagents, with government laboratory research efforts of the Army, Navy, Air Force, and Marines has expedited development and validation of this surveillance and diagnostic system.

## Materials and Methods

The JBAIDS Validation Testing (JVT) is one evaluation in a comprehensive line of testing for the system. Assays designed and developed by Government laboratories, Idaho Technology Inc. (ITI), and Midwest Research Institute (MRI) were initially converted to a formulation suitable for freeze-drying, and re-optimized and evaluated through an Assay Development/Assessment (ADA) process. Thirty of the most sensitive assays were chosen to move forward from ADA into the JVT testing procedure (Table 1), as well as into stability studies for the freeze-dried reagents.

Table 1: Assays in JVT

Disease	Assays Needed	Assays Evaluated	Reagent Age at Testing
Anthrax	2	3	6 months
Smallpox	1	2	6 months
Orthopox	1	2	6 months
Plague	2	3	6 months
Tularemia	1	2	6 months
Brucellosis	1	2	6 months
Q Fever	1	2	6 months
Glanders	1	2	6 months
Typhus	1	2	6 months
Viral Encephalitis	3	6	0.5 months
Hemorrhagic Fever	2	4	0.5 months

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## Results

All 30 assays evaluated in JVT passed the specifications with 85% success at 85% or 95% confidence, depending on the matrix (see table 2 for confidence information). Data for a representative DNA assay (Brucella spp.) is shown comparing initial and 6 month Crossing Threshold and Max Fluorescence from the JBAIDS platform (Table 4). Amplification curves from the JBAIDS analysis show that the curves are unchanged after reagents are stored 6 months at room temperature (Figure 1). Data for a representative RNA virus assay (Marburg) is shown, but only the initial time point (Table 5 and Figure 2). Studies are on going for the RNA virus assays. Ongoing stability studies suggest that 1 year shelf-life is likely.

Table 4: Brucella spp. Assay Initial and 6 Month Timepoints

Matrix	Reaction/Sample Type	Number of Samples Tested	Mean Crossing Threshold Initial(%CV)	Mean Crossing Threshold 6 months(%CV)	Mean Maximum Fluorescence Initial(%CV)	Mean Maximum Fluorescence 6 months(%CV)
Blood	PTC	3	30.72 (1.18)	30.19 (2.88)	11.75 (29.71)	17.72 (5.52)
	100 cfu/ml	6	37.13 (2.13)	36.22 (3.48)	3.43 (104.5)	2.82 (110.46)
	1000 cfu/ml	30	34.8 (1.6)	34.72 (2.38)	9.97 (12.23)	12.84 (12.4)
	10000 cfu/ml	3	31.04 (0.39)	31.01 (1.23)	9.6 (9.07)	12.74 (6.73)
	Matrix Blanks	3	-	-	0.03 (27.08)	0.13 (37.19)
Culture	PTC	3	30.37 (1.84)	30.85 (2.34)	12.94 (13.02)	18.14 (10.25)
	100 cfu/ml	9	37.52 (4.78)	38.21 (5.63)	6.99 (71.55)	11.10 (57.17)
	1000 cfu/ml	26	34.71 (6.37)	35.06 (2.88)	12.41 (23.26)	15.89 (10.18)
	10000 cfu/ml	4	31.92 (1.54)	31.62 (2.03)	15.18 (4.9)	22.73 (22.71)
	Matrix Blanks	3	-	-	0.04 (39.98)	0.03 (46.92)
PBS	PTC	3	30.87 (2.4)	31.08 (0.44)	12.11 (7.7)	18.10 (10.33)
	100 cfu/ml	6	40.43 (6.77)	39.24 (4.79)	7.21 (89.27)	7.15 (106.13)
	1000 cfu/ml	30	36.75 (3.42)	36.77 (1.34)	7.91 (87.18)	14.89 (10.27)
	10000 cfu/ml	3	33.89 (1.57)	33.48 (1.09)	18.71 (4.7)	18.26 (10.26)
	Matrix Blanks	3	-	-	0.04 (83.47)	0.04 (27.55)
Powder	PTC	3	29.15 (6.13)	30.24 (2.89)	10.21 (26)	18.20 (17.40)
	100 cfu/ml	9	36.76 (3.48)	36.96 (3.39)	9.88 (28.37)	12.55 (37.08)
	1000 cfu/ml	26	33.36 (3.24)	33.87 (1.69)	13.28 (14.64)	15.56 (24.38)
	10000 cfu/ml	4	31.06 (2.03)	30.48 (0.88)	12.79 (6.86)	17.61 (5.13)
	Matrix Blanks	3	-	-	0.04 (56.65)	0.02 (44.23)
Nasal Swab	PTC	3	30.61 (2.2)	30.97 (2.14)	17.73 (4.3)	18.05 (5.73)
	100 cfu/ml	6	37.71 (6.01)	40.64 (6.11)	3.87 (104.1)	4.39 (175.66)
	1000 cfu/ml	30	35 (3.74)	37.01 (4.80)	15.93 (12.64)	14.72 (18.16)
	10000 cfu/ml	3	32.93 (1.44)	31.01 (1.12)	19.15 (6.63)	12.28 (76.18)
	Matrix Blanks	3	-	-	0.04 (32.29)	0.04 (47.65)
Surface Swab	PTC	3	30.45 (4.64)	30.6 (1.52)	16.82 (10.23)	17.95 (5.08)
	100 cfu/ml	9	41.59 (3.72)	40.08 (6.04)	5.63 (133.9)	5.72 (111.10)
	1000 cfu/ml	26	36.69 (4.3)	36.75 (5.02)	14.23 (10.29)	14.89 (15.69)
	10000 cfu/ml	4	33.91 (2.09)	32.98 (1.86)	18.33 (8.61)	18.70 (10.63)
	Matrix Blanks	3	-	-	0.05 (39.19)	0.04 (38.65)

Figure 1: Brucella spp. Assay Initial and 6 Month Analysis Curves

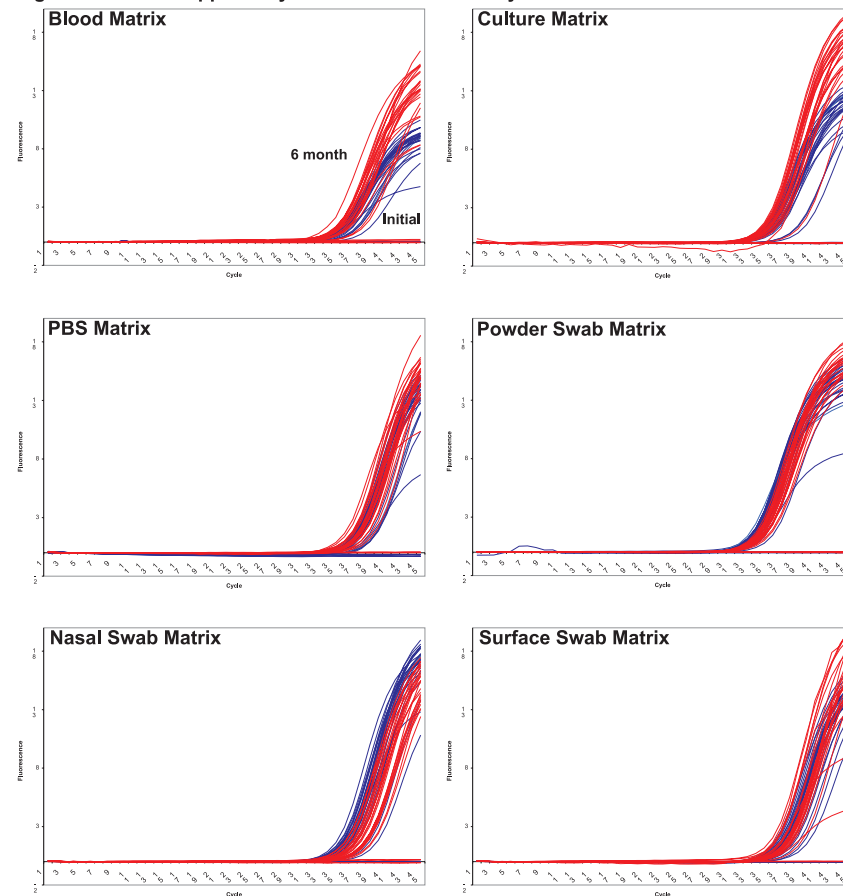
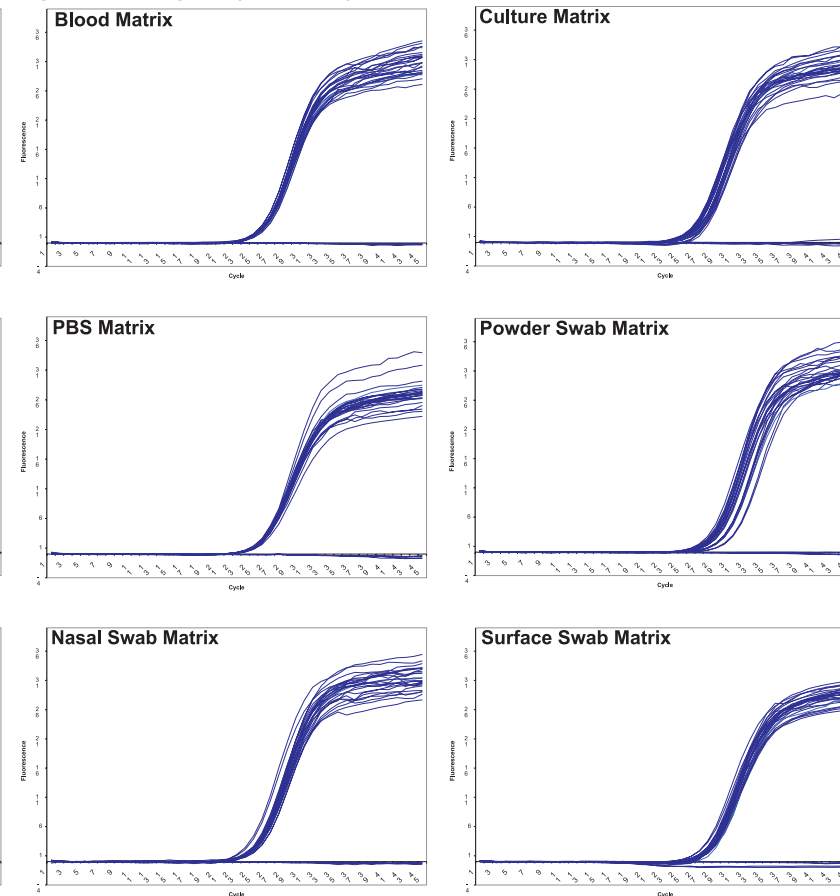


Table 5: Marburg Assay Initial JVT Data

Matrix	Reaction/Sample Type	Number of Samples Tested	Mean Crossing Threshold	%CV Crossing Threshold	Mean Maximum Fluorescence	%CV Maximum Fluorescence
Blood	PTC	3	27.76	0.84	30.57	4.69
	100 pfu/ml	6	27.42	1.17	29.68	5.25
	1000 pfu/ml	30	25.02	3.01	31.35	6.28
	Matrix Blanks	3	-	-	0.17	24.94
	NTC	3	-	-	0.14	12.43
Culture	PTC	3	27.66	0.64	31.29	2.19
	100 pfu/ml	9	30.55	0.69	26.46	5.22
	1000 pfu/ml	26	26.35	2.62	29.76	6.46
	Matrix Blanks	3	-	-	0.27	79.77
	NTC	3	-	-	0.32	97.69
PBS	PTC	3	27.46	0.28	28.5	3.95
	100 pfu/ml	6	28.31	0.82	25.28	6.59
	1000 pfu/ml	30	25.97	3.53	26.54	8.22
	Matrix Blanks	3	-	-	0.13	14.57
	NTC	3	-	-	0.13	76.37
Powder	PTC	3	27.61	0.39	31.26	2.74
	100 pfu/ml	9	31.04	3.43	27.06	6.06
	1000 pfu/ml	26	28.91	3.73	30.51	8.74
	Matrix Blanks	3	-	-	0.19	35.88
	NTC	3	-	-	0.18	29.05
Nasal Swab	PTC	3	27.56	0.44	31.10	3.37
	100 pfu/ml	6	27.74	4.77	36.52	7.16
	1000 pfu/ml	30	23.78	3.29	32.22	7.27
	Matrix Blanks	3	-	-	0.15	35.49
	NTC	3	-	-	0.15	48.45
Surface Swab	PTC	3	27.49	0.17	30.93	6.32
	100 pfu/ml	9	32.21	0.61	22.55	12.59
	1000 pfu/ml	26	26.96	1.27	27.92	7.05
	Matrix Blanks	3	-	-	0.09	31.54
	NTC	3	-	-	0.11	33.95

Figure 2: Marburg Assay Initial Analysis Curves



## Materials and Methods (continued)

The JVT incorporates sample purification using specified kits and protocols, PCR or Reverse Transcription (RT)-PCR amplification of the purified template using freeze-dried reagents on the JBAIDS, and ultimately automated analysis of the data generated.

The first part of JVT consists of the spiking of various matrices with specified levels of quantified inactivated organism (IO), provided by the Department of Defense (DoD) Critical Reagents Program, and isolating the DNA or RNA from these spiked specimens. The six matrices are spiked with various concentrations of IO depending on the type of matrix (Table 2). These samples are processed using the appropriate IT1-2-3 kit and protocol (Table 2).

Table 2: Matrices and Sample Concentrations

Matrix	IT 1-2-3 Kit	Limit Of Detection (LOD) Specification	Amount Processed/sample
Blood *	FLOW	1000 cfu/ml or 10,000 pfu/ml	3 ml
PBS (from air)*	FLOW	1000 cfu/ml or 10,000 pfu/ml	5 ml
Culture	SWIPE	1000 cfu/ml or 10,000 pfu/ml	40 µl
Powder	SWIPE	1000 cfu/ µgor 10,000 pfu/ µg	10 µg
Nasal Swab*	SWIPE	1000 cfu/swab or 10,000 pfu/swab	1 swab
Surface Swab	SWIPE	1000 cfu/swab or 10,000 pfu/swab	1 swab

\*These matrices were tested at 95% confidence.

The purified samples are then added to the freeze-dried reagent tube along with Reconstitution Buffer. Once reconstituted, the freeze-dried reaction contains all necessary components for the PCR or RT-PCR to be performed on the JBAIDS platform. The JBAIDS platform is programmed using a software Wizard that directs a thermocycling protocol, and an analysis protocol based on the target selected. At the end of cycling the JBAIDS software analyzes the real-time data and automatically generates a "Positive", "Negative", or "Uncertain" result for each sample.

The JVT also includes specificity testing on organisms that are related to the disease-causing organism of interest. Nearest neighbors are tested at LOD levels of purified DNA or RNA template also provided by the government Critical Reagents Program. The nearest neighbor targets for the JVT diseases of interest are shown in Table 3.

Table 3: Nearest Neighbors

Disease	Nearest Neighbor Target
Anthrax	Bacillus thuringiensis
Smallpox	Vaccinia
Orthopox	Herpes Simplex Virus
Plague	Yersinia enterocolitica
Tularemia	Yersinia pseudotuberculosis
Brucellosis	Pseudomonas aeruginosa
Q Fever	Rickettsia prowazekii
Glanders	Pseudomonas aeruginosa
Typhus	Coxiella burnetii
Viral Encephalitis	related Viral Encephalitis
Hemorrhagic Fever	Rubella Virus

## Conclusions

The real-time PCR JBAIDS platform, coupled with defined sample extraction kits and room temperature stable freeze dried reagents, shows equivalent performance levels at initial testing and 6 month storage for all DNA agents/assays. All 30 assays evaluated passed the JVT specifications with 85% success at 85% or 95% confidence in all of their respective relevant matrices. The freeze-dried assays designed to detect DNA targets have been shown to be stable at room temperature for at least 6 months at 28°C, and ongoing studies suggest that the reagents will be stable out to 1-year. Similar stability studies are in progress for assays designed to detect RNA viral targets. Sixteen assays that exhibited the best overall sensitivity, specificity and stability, have been jointly selected by the JBAIDS Program Office, Idaho Technology and Midwest Research Institute, to be the JBAIDS assays.

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