

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

K131936

**B. Purpose for Submission:**

The purpose of this submission is to add the IT 1-2-3 Platinum Path Sample Purification Kit as an accessory to the cleared JBAIDS Tularemia Detection Kit (K072547) for the purification of DNA from whole blood, sputum, and colony specimens.

**C. Measurand:**

*Francisella Tularensis* DNA sequences

**D. Type of Test:**

A real-time polymerase chain reaction (PCR) test kit intended for the qualitative *in vitro* diagnostic (IVD) detection of target DNA sequences of *Francisella tularensis* (*F. tularensis*).

**E. Applicant:**

BioFire Diagnostics, Inc.

**F. Proprietary and Established Names:**

**Trade Name:** Joint Biological Agent Identification and Diagnostic System (JBAIDS)  
Tularemia Detection Kit

**Common Name:** Real-time PCR amplification and detection system for targeted *F. tularensis* DNA sequences

**G. Regulatory Information:**

1. Regulation section:

21 CFR § 866.3280, *Francisella tularensis* Serological Reagents

2. Classification:

Class II

3. Product code:

OEH

4. Panel:

Microbiology (83)

**H. Intended Use:**

1. Intended use(s):

The Joint Biological Agent Identification and Diagnostic System (JBAIDS) Tularemia Detection Kit is a real-time polymerase chain reaction (PCR) test kit intended for the qualitative *in vitro* diagnostic (IVD) detection of target DNA sequences of *Francisella tularensis*. The system can be used to test human whole blood collected in sodium citrate or sputum collected aseptically from individuals greater than 18 years of age suspected of having tularemia. In addition, positive blood cultures and colonies may be tested. This assay is intended to aid in the diagnosis of individual presenting with signs and symptoms of pneumonic or typhoidal tularemia. It is not intended to aid in the diagnosis of glandular, ulceroglandular, oculoglandular, or oropharyngeal tularemia.

The JBAIDS Tularemia Detection Kit is run on the JBAIDS instrument using the Diagnostic Wizard. Results are for the presumptive identification of *F. tularensis* in conjunction with culture and other laboratory tests. The definitive identification of *F. tularensis* from colony growth, liquid blood culture growth, blood specimens, or sputum specimens requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reports are required.

The diagnosis of tularemia must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the identification of the target either from colonies, blood culture, whole blood or sputum specimens.

**The JBAIDS Tularemia Detection Kit is intended for use by trained clinical laboratory personnel who have received specific training on the use of the JBAIDS Tularemia Detection Kit. The level of *F. tularensis* that would be present in blood or sputum from individuals with early systemic or pneumonic infection is unknown. Due to the difficulty in obtaining clinical specimens, this assay was not evaluated with blood or sputum from individuals presenting with signs and symptoms of tularemia who have subsequently developed pneumonic or typhoidal tularemia.**

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

The JBAIDS Tularemia Detection Kit is intended for use by trained clinical laboratory personnel who have received specific training on the use of the JBAIDS Tularemia Detection Kit. The level of *F. tularensis* that would be present in blood or sputum of

individuals with early systemic or pneumonic infection is unknown. Due to the difficulty in obtaining clinical specimens, the assay was not evaluated with blood or sputum from individuals presenting with signs and symptoms of tularemia and who subsequently developed pneumonic or typhoidal tularemia.

4. Special instrument requirements:

JBAIDS instrument

**I. Device Description:**

**Note:** The JBAIDS Tularemia Detection Kit (K072547) was developed by Idaho Technology earlier. Now it is produced by BioFire Diagnostics.

The JBAIDS Tularemia Detection System is composed of the portable JBAIDS instrument, laptop computer and software, and the JBAIDS Tularemia Detection Kit, which includes specific freeze-dried reagents for the detection of a gene target of the *F. tularensis*. The JBAIDS Tularemia Detection Kit is specially designed for performing real-time PCR in glass capillaries using the JBAIDS instrument and JBAIDS software. The JBAIDS Tularemia Detection System was fully described earlier, please refer to FDA cleared 510(k) submission: K072547.

The JBAIDS Tularemia Detection Kit (K072547) was previously cleared for use with three different sample purification kits i.e., IT 1-2-3 QFLOW<sup>dna</sup> for whole blood, IT 1-2-3 VIBE for sputum, and IT 1-2-3 SWIPE for direct culture from blood agar Plate and positive blood culture media.

The purpose of this 510(k) submission was to add the IT 1-2-3<sup>TM</sup> Platinum Path Sample Purification Kit as the sample purification kit for use with the previously cleared JBAIDS Tularemia Detection Kit. The IT 1-2-3 Platinum Path Sample Purification Kit uses magnetic bead technology to isolate nucleic acids, and the kit has been optimized for extraction of nucleic acids from a wide variety of sample types. The IT 1-2-3 Platinum Path Sample Purification Kit can replace all of the other sample purification kits used in the JBAIDS program. The purpose of addition of IT 1-2-3<sup>TM</sup> Platinum Path Sample Purification Kit was to reduce and simplify the supplies and reagents to use the system in military arenas.

The JBAIDS Tularemia Detection System has now been validated using four different sample preparation kits for isolating DNA from whole blood, sputum, direct culture from blood agar Plate and positive blood culture media. The table below shows the comparison of four IT 1-2-3 Sample Purification Kits Validated for use with the JBAIDS Tularemia Detection Kit.

**Comparison of the Four IT 1-2-3 Sample Purification Kits Validated for use with the JBAIDS Tularemia Detection Kit**

Clinical Specimens	IT 1-2-3 Sample Purification Kit	Approximate Time to Purify 1-6 Samples	Technologies Used
Whole Blood	Platinum Path	60 minutes	Mechanical and chemical lysis Magnetic bead separation (no centrifugation)
	QFLOW <sup>dna</sup>	90-120 minutes	Mechanical and chemical lysis Spin-filter centrifugation (small centrifuge for 1.5 mL tubes)
Sputum	Platinum Path	90 minutes	Mechanical and chemical lysis Magnetic bead separation (no centrifugation)
	VIBE	120 minutes	Mechanical and chemical lysis Spin-filter centrifugation (small centrifuge for 1.5 mL tubes)
Colonies (Direct Culture from Plates)	Platinum Path	15 minutes	Mechanical lysis
	SWIPE	15 minutes	Mechanical lysis
Positive Blood Culture Matrix	SWIPE	15 minutes	Mechanical lysis

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

JBAIDS Tularemia Detection Kit

2. Predicate 510(k) number(s):

K072547

3. Comparison with predicate:

### Similarities between the New Device and the Predicate

Element	New Device: JBAIDS Tularemia Detection Kit with addition of Platinum Path Sample Purification Kit	Predicate: JBAIDS Tularemia Detection Kit (K072547)
Intended Use	Presumptive identification of Tularemia infection through the detection of a DNA sequence unique to <i>Francisella tularensis</i> . Results are used in conjunction with clinical information, culture, and other laboratory tests as an aid in the diagnosis individuals presenting with signs and symptoms of pneumonic or typhoidal tularemia.	Same
Organism Detected	Qualitative <i>in vitro</i> detection of <i>Francisella tularensis</i> DNA	Same
Technology	Real-time PCR using hydrolysis probes	Same
Specimen Types	Whole blood (collected in 3.2% sodium citrate), sputum collected aseptically from individuals greater than 18 years of age suspected of having tularemia, blood culture (grown in soybean-casein digest broth) or bacterial culture (grown on blood agar)	Same
Technological Principles	Nested multiplex RT-PCR followed by high resolution melting analysis to confirm identity of amplified product.	Same
Platform	JBAIDS Instrument	Same
Time Required for Analysis of Specimen	Less than 3 hours	Same

**Differences between the New Device and the Predicate**

Element	New Device: JBAIDS Tularemia Detection Kit with addition of Platinum Path Sample Purification Kit	Predicate: JBAIDS Tularemia Detection Kit (K072547)
DNA Extraction Methods	Whole blood purified with IT 1-2-3™ Platinum Path or IT 1-2-3™ QFLOW DNA Sample Purification Kits (or validated equivalent).	Whole blood purified with IT 1-2-3™ QFLOW DNA Sample Purification Kit (or validated equivalent).
	Sputum purified with IT 1-2-3™ Platinum Path or IT 1-2-3™ VIBE Sample Purification Kits (or validated equivalent).	Sputum purified with IT 1-2-3™ VIBE Sample Purification Kits (or validated equivalent).
Platform	Blood culture purified with IT 1-2-3™ SWIPE Sample Purification Kit (or validated equivalent).	Same
Time Required for Analysis of Specimen	Direct bacterial culture purified with IT 1-2-3™ Platinum Path or IT 1-2-3™ SWIPE Sample Purification Kit (or validated equivalent).	Direct bacterial culture purified with IT 1-2-3™ SWIPE Sample Purification Kit (or validated equivalent).

**K. Standard/Guidance Document Reference (if applicable):**

1. Molecular Diagnostic Methods for Infectious Diseases,” CLSI Approved Guideline, MM3-A2 (February 2006).
2. Evaluation of Precision Performance of Quantitative Measurements Methods; Approved Guidance-Second Edition”, CLSI Approved Guidance EP5-A2 (August 2004).
3. Protocols for Determination of Limits of Detection and Limits of Quantitation, CLSI Approved Guidance EP17-A (2004).

**L. Test Principle:**

Refer to previously FDA-cleared 510(k) Premarket Notification: K072547

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:
  - a. *Precision/Reproducibility:*

The reproducibility of JBAIDS Tularemia Detection Kit in conjunction with the Platinum Path Sample Purification Kit was evaluated by testing a panel of whole blood and sputum samples described below.

The evaluation with whole blood samples was conducted by testing one panel of twelve pooled human whole blood samples twice each day for four days at each of three testing sites. Spiking was performed at levels relative to the newly established LoD of 1500 CFU/mL. Specifically, the panel contained four samples spiked with inactivated *F. tularensis* at a medium positive (5×LoD) level, four samples spiked at a low positive level (1×LoD), and four samples that were not spiked. The samples were prepared at BioFire Diagnostics, aliquoted and stored at 2-8°C for testing at BioFire Diagnostics or shipped overnight on ice for external site testing.

The evaluation with sputum samples was conducted by testing one panel of nine pooled residual sputum samples twice each day for five days at each of three testing sites. The panel contained three samples spiked with inactivated *F. tularensis* at a medium positive (5×LoD) level, three samples spiked at a low positive level (1×LoD), and three samples that were not spiked. The samples were prepared at BioFire Diagnostics, aliquoted and stored frozen ( $\leq 15^{\circ}\text{C}$ ) for testing at BioFire Diagnostics or shipped frozen on dry ice for external site testing.

On each testing day, two users at each site purified and tested one aliquot of each sample in the panel under evaluation. A total of 96 whole blood sample replicates and 90 sputum sample replicates tested at each analyte level. All samples were processed with the IT 1-2-3 Platinum Path Sample Purification Kit.

Overall for whole blood and sputum testing, system variability as measured by the coefficient of variation (% CV) was 2-4% for samples spiked at the medium positive (5×LoD) level, and 5-7% for samples spiked at the low positive (LoD) level across test sites.

The results of the reproducibility evaluation indicate that the JBAIDS Tularemia Detection System is reproducible when used in conjunction with the IT 1-2-3 Platinum Path Sample Purification Kit for human whole blood and sputum samples. The performance of the system is not significantly affected by the variability associated with different samples, operators, instruments, and test sites. The reproducibility results for whole blood and sputum samples are acceptable and presented below in the tables.

**Reproducibility of the Tularemia Target Assay in the JBAIDS Tularemia Detection Kit for Whole Blood Samples Purified with the IT 1-2-3 Platinum Path Purification Kit**

Blood Spike Level	Test Location	Tularemia Target Assay							
		Number Positive	Number Uncertain	Number Negative	% Agreement with Expected Result	95% CI	Mean Cp <sup>a</sup>	Std Dev	%CV
<b>Medium Positive (5xLoD)</b>	Site 1	32/32	0/32	0/32	100%		35.16	0.77	2.19
	Site 2	32/32	0/32	0/32	100%		35.83	1.40	3.91
	Site 3	32/32	0/32	0/32	100%		33.88	1.34	3.96
	<b>All Sites</b>	<b>96/96</b>	<b>0/96</b>	<b>0/96</b>	<b>100%</b>	<b>96.2-100</b>	<b>35.00</b>	<b>1.43</b>	<b>4.09</b>
<b>Low Positive (1xLoD)</b>	Site 1	28 <sup>b</sup> /32	0/32	4/32 <sup>c</sup>	87.5%		38.47	2.31	6.00
	Site 2	24 <sup>d</sup> /32	5 <sup>e</sup> /32	3/32	75%		39.38	2.32	5.89
	Site 3	31 <sup>f</sup> /32	0/32	1/32	96.9%		36.53	2.32	6.35
	<b>All Sites</b>	<b>83/96</b>	<b>5/96</b>	<b>8/96</b>	<b>86.5%</b>	<b>78.0-92.6</b>	<b>38.13</b>	<b>2.59</b>	<b>6.79</b>
<b>Negative</b>	Site 1	0/32	0/32	32/32	100%				
	Site 2	0/32	0/32	32/32	100%				
	Site 3	0/32	0/32	32/32	100%				
	<b>All Sites</b>	<b>0/96</b>	<b>0/96</b>	<b>96/96</b>	<b>100%</b>	<b>96.2-100</b>			

<sup>a</sup> Cp values included for the samples that amplified only.

<sup>b</sup> Three results were initially uncertain but were positive when retested.

<sup>c</sup> Two results were initially uncertain but were negative when retested.

<sup>d</sup> Eight results were initially uncertain but were positive when retested.

<sup>e</sup> Five results were initially uncertain and were uncertain when retested.

<sup>f</sup> Three results were initially uncertain but were positive when retested.

**Reproducibility of the Tularemia Target Assay in the JBAIDS Tularemia Detection Kit for Sputum Samples Purified with the IT 1-2-3 Platinum Path Purification Kit**

Sputum Spike Level	Test Location	Tularemia Target Assay							
		Number Positive	Number Uncertain	Number Negative	% Agreement with Expected Result	95% CI	Mean Cp <sup>a</sup>	Std Dev	%CV
<b>Medium Positive (5xLoD)</b>	Site 1	30/30	0/30	0/30	100%		35.35	1.46	4.13
	Site 2	30/30	0/30	0/30	100%		34.65	0.66	1.90
	Site 3	30/30	0/30	0/30	100%		34.37	0.53	1.54
	<b>All Sites</b>	<b>90/90</b>	<b>0/90</b>	<b>0/90</b>	<b>100%</b>	<b>96.0-100</b>	<b>34.79</b>	<b>1.05</b>	<b>3.02</b>
<b>Low Positive (1xLoD)</b>	Site 1	28 <sup>b</sup> /30	1 <sup>c</sup> /30	1/30	93.3%		39.19	2.62	6.69
	Site 2	30 <sup>d</sup> /30	0/30	0/30	100%		38.49	2.37	6.16
	Site 3	30 <sup>e</sup> /30	0/30	0/30	100%		38.15	2.22	5.82
	<b>All Sites</b>	<b>88/90</b>	<b>1/90</b>	<b>1/90</b>	<b>97.8%</b>	<b>92.2-99.7</b>	<b>38.60</b>	<b>2.43</b>	<b>6.30</b>
<b>Negative</b>	Site 1	0/30	0/30	30/30	100%				
	Site 2	0/30	0/30	30/30	100%				
	Site 3	0/30	0/30	30/30	100%				
	<b>All Sites</b>	<b>0/90</b>	<b>0/90</b>	<b>90/90</b>	<b>100%</b>	<b>96.0-100</b>			

<sup>a</sup> Cp values included for the samples that amplified only.

<sup>b</sup> Four results were initially uncertain but were positive when retested.

<sup>c</sup> One result was initially uncertain and was uncertain when retested.

<sup>d</sup> Two results were initially uncertain but were positive when retested.

<sup>e</sup> One result was initially uncertain and was positive when retested.

*b. Linearity/assay reportable range:*

Not applicable.

*c. Traceability, Stability, Expected values (controls, calibrators, or methods):*

There are no changes made to the controls for the assay. Please refer to previously FDA-cleared 510(k) Premarket Notification K072547.

*d. Detection limits:*

The Limit of Detection (LoD) for the JBAIDS Tularemia Detection Kit was determined with the newer IT 1-2-3™ Platinum Path Sample Purification Kit for whole blood and sputum samples. The LoD testing was performed in two phases using live *F. tularensis* as described below.

Part I: Comparison of Sample Purification Kits

Initial estimates for the LoD were performed by spiking whole or sputum specimens with a serial dilution of live *F. tularensis* at 10x, 1x, and 0.1x the previously established LoD (300 CFU/mL for whole blood, 2000 CFU/mL for sputum). The samples were then split and purified in parallel using both purification methods *i. e.*, Whole blood purified with IT 1-2-3™ Platinum Path or IT 1-2-3™ QFLOW<sup>DNA</sup> Sample Purification Kits or

Sputum purified with IT 1-2-3™ Platinum Path or IT 1-2-3™ VIBE Sample Purification Kits.

**Part II: Confirmation of the Platinum Path Sample LoDs**

The LoD of the whole blood and sputum for the JBAIDS Tularemia Detection Kit was further confirmed by purifying 20 individually prepared whole blood spiked samples and 20 individually prepared sputum spiked samples with *F. tularensis*, followed by purification only with the Platinum Path kit.

Initial direct comparison between the QFLOW<sup>DNA</sup> and Platinum Path whole blood purification methods showed the similar LoD of 300 CFU/mL but it was not confirmed in all 20 individually prepared whole blood spiked samples (detection was 8/20 or 40%). To reset the LoD for whole blood purified with the Platinum Path kit, a refinement dilution series at the 50x, 5x, and 0.5x LoD levels was tested and confirmed. The LoD for whole blood samples purified with the Platinum Path Sample purification kit was confirmed at 1500 CFU/mL.

There was no difference in the LoD of the sputum specimens using the QFLOW<sup>DNA</sup> and Platinum Path sputum purification methods. The original LoD for sputum samples was confirmed (detection was 19/20 or 95%) at 2000 CFU/mL with the Platinum Path kit.

The table below shows the LoD for Platinum Path-purified whole blood and sputum samples tested with the JBAIDS Tularemia Detection Kit.

**LoDs for Platinum Path-Purified Whole Blood and Sputum Samples Tested with the JBAIDS Tularemia Detection Kit**

Sample Matrix	Spiked <i>F. tularensis</i> Concentration (CFU/mL)	# Positive	% Positive	Tularemia Target Assay Mean Cp +/- Std Dev
Whole Blood	1500	20/20	100.0%	36.50 ± 1.81
Sputum	2000	19/20	95.0%	36.47 ± 1.70

*Additional Studies: Detection of Direct Culture Samples Processed with the IT 1-2-3 Platinum Path Sample Purification Kit*

The JBAIDS Tularemia Detection Kit was evaluated to detect and identify *F. tularensis* from direct culture samples (colonies) processed using a modified IT 1-2-3 Platinum Path Sample Purification Kit protocol. The direct culture sample processing procedure and reagents are identical for the modified Platinum Path and the previously validated SWIPE protocol [that was previously FDA-cleared for use with the JBAIDS Tularemia Detection Kit (K072547)], except that intermediate reagent volumes vary. The modified Platinum Path protocol for processing direct culture samples was designed to deliver the same amount of PCR template to a reaction as the SWIPE protocol. For both kits, the colony is processed by mechanical lysis followed by dilution in an elution buffer.

This study was conducted using *F. tularensis* Schu4 strain colonies to demonstrate that the test system accurately detects *F. tularensis*. Colonies of *B. anthracis* and *Y. pestis* were tested to demonstrate negative results. Each organism was grown on separate agar plates. Once colonies were at least 1.5 mm in diameter, 10 individual positive *F. tularensis* colonies and 10 individual non-*F. tularensis* colonies (five from the *B. anthracis* cultures and five from the *Y. pestis* cultures) were processed using the Platinum Path “Direct testing of Bacterial Cultures Protocol” and tested with the JBAIDS Tularemia Detection Kit. Since the number of colony forming units (CFU) typically sampled from a colony is exceedingly high, qualitative results were expected to be positive, with early Cp values.

All samples containing *F. tularensis* yielded positive results, and no *F. tularensis* was detected in any of the negative control samples (of *B. anthracis* or *Y. pestis*). The Target Cp values for the *F. tularensis* colonies were fifteen cycles earlier than Cp values for whole blood samples spiked at the LoD and purified by Platinum Path.

The JBAIDS Tularemia Detection Kit assay is capable of detecting and identifying *F. tularensis* from direct culture samples processed using a modified IT 1-2-3 Platinum Path Sample Purification Kit. Results for the testing of ten *F. tularensis* colonies and ten non-*F. tularensis* colonies are shown in the table below.

**Tularemia Target Detection from Colonies Purified with Platinum Path**

Colony Type	Tularemia Target		
	Positive Results/Total	Cp (cycles)	
		Mean	SD
<i>F. tularensis</i>	10/10	21.00	0.31
Non- <i>F. tularensis</i>	0/10	-	-

e. *Analytical reactivity:*

Refer to previously FDA-cleared 510(k) Premarket Notifications K072547.

f. *Analytical specificity:*

Refer to previously FDA-cleared 510(k) Premarket Notifications K072547.

g. *Competitive interference:*

Refer to previously FDA-cleared 510(k) Premarket Notifications K072547.

2. Comparison studies:

**Clinical Testing**

Two clinical evaluations were performed to validate the use of the IT 1-2-3™ Platinum Path Sample Purification Kit to process clinical specimens with the JBAIDS Tularemia Detection Kit. True clinical specimens from patients infected with *Francisella tularensis*

(tularemia), are not available for testing due to the extreme rarity of natural infection with this organism. Therefore, two clinical evaluations using surrogate specimens were performed to validate the use of the IT 1-2-3™ Platinum Path Sample Purification Kit with the JBAIDS Tularemia Detection Kit.

The first clinical valuation used prospectively collected whole blood specimens collected in sodium citrate and obtained from patients with febrile illness. The second clinical evaluation used residual frozen sputum specimens that were spiked with inactivated *F. tularensis*. In both evaluations, the spiked samples were purified in parallel using the new and old extraction methods, and then tested with the JBAIDS Tularemia Detection Kit.

### ***Testing of Surrogate Whole Blood Clinical Specimens***

One hundred (100) surrogate whole blood specimens were prepared using prospectively collected specimens that were collected from febrile volunteers from November of 2012 into April of 2013. Fifty (50) of the specimens were spiked with inactivated *F. tularensis* at concentrations near and above the system LoD, while the remaining 50 specimens were not spiked with *F. tularensis*. The level of inactivated *F. tularensis* used to spike these samples was relative to the LoD (1500 CFU/mL) established for Platinum Path-purified whole blood specimens.

Once spiked, samples were then processed using both the new nucleic acid extraction method (Platinum Path) and the original nucleic acid extraction method (IT 1-2-3™ QFLOW<sup>DNA</sup> Sample Purification Kit; QFLOW<sup>DNA</sup>) followed by testing with the JBAIDS Tularemia Detection Kit. JBAIDS operators were blinded to the analyte content of the samples. The results obtained with the Platinum Path processed samples were compared to the results obtained with the QFLOW<sup>DNA</sup> processed samples.

The JBAIDS result for a sample purified using from the QFLOW<sup>DNA</sup> Kit was considered the correct result. The mean Cp values decrease as the analyte concentrations increase. The table below provides the JBAIDS Tularemia test results and Cp values stratified by *F. tularensis* spike level.

**Cp Analysis of JBAIDS Tularemia Detection Kit Testing of Spiked Whole Blood Samples Processed with the IT 1-2-3 Platinum Path and QFLOW<sup>DNA</sup> Sample Purification Kits**

<i>F. tularensis</i> Spike Level	Sample Purification Kit					
	Platinum Path			QFLOW <sup>DNA</sup>		
	JBAIDS Positive/ Total	Mean Cp	SD	JBAIDS Positive/ Total	Mean Cp	SD
No spike	0/50	-	-	0/50	-	-
1 × LoD	20/20	35.14	1.06	20/20	32.44	0.94
5 × LoD	10/10	32.78	0.44	10/10	29.45	0.82
10 × LoD	10/10	32.48	1.16	10/10	28.72	0.87
100 × LoD	5/5	28.75	0.92	5/5	25.62	0.74
1,000 × LoD	5/5	27.95	0.51	5/5	22.80	1.03
≥ LoD	<b>50/50</b>	-	-	<b>50/50</b>	-	-

The final JBAIDS Tularemia interpretation for samples purified using the Platinum Path Sample Purification Kit had a positive percent agreement (PPA) of 100% as compared to samples purified using the QFLOW<sup>DNA</sup> kit (50/50; 95% CI = 92.9-100%). The final JBAIDS Tularemia interpretation for samples purified using Platinum Path Sample Purification Kit was negative for 50 out of 50 samples that were negative when purified using QFLOW<sup>DNA</sup>. This represents a negative percent agreement (NPA) of 100% (50/50; 95% CI = 92.9-100%). Using samples spiked near the LoD for samples purified with the Platinum Path kit (1500 CFU/mL), the IT 1-2-3 QFLOW<sup>DNA</sup> and Platinum Path Sample Purification Kits performed equivalently with respect to detection of *F. tularensis* in surrogate whole blood specimens tested with the JBAIDS Tularemia Detection Kit. The table below presents the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for the surrogate whole blood specimen testing.

**JBAIDS Tularemia Detection Kit Performance on Spiked Whole Blood Samples Processed with the IT 1-2-3 Platinum Path and QFLOW<sup>DNA</sup> Sample Purification Kits**

Positive Agreement				Negative Agreement			
QFLOW + Platinum Path +	QFLOW + Platinum Path -	PPA	95% CI	QFLOW - Platinum Path -	QFLOW - Platinum Path +	NPA	95% CI
50	0	100% (50/50)	92.9- 100%	50	0	100% (50/50)	92.9- 100%

**Testing of Surrogate Sputum Clinical Specimens**

One hundred (100) surrogate specimens were prepared using frozen residual sputum specimens. Fifty (50) of the specimens were spiked with inactivated *F. tularensis* at concentrations near and above the system LoD, while the remaining 50 specimens were not spiked with *F. tularensis*.

**Sputum Sample Spike Levels and Replicate Numbers**

<i>F. tularensis</i> Spike Level	Live Organism in CFU/mL (Inactivated Organism Equivalent) <sup>a</sup>	Number of Sample Replicates Processed with Each Sample Purification Kit
No spike	–	50
1 × LoD	2000 (20,000)	20
5 × LoD	10,000 (100,000)	10
10 × LoD	20,000 (200,000)	10
100 × LoD	200,000 (2,000,000)	5
1,000 × LoD	2,000,000 (20,000,000)	5
<b>Total</b>		<b>100</b>

<sup>a</sup> Samples were spiked with inactivated organism at concentrations that were adjusted to match the PCR performance of live organism. The initial estimated inactivated organism equivalent was shown to be too low when tested with high numbers of replicate samples spiked with inactivated organism at the 1 × LoD level. Therefore the inactivated organism equivalent at the 1 × LoD level was renormalized to match the performance of live organism.

Samples were then processed using both the new nucleic acid extraction method (Platinum Path) and the original nucleic acid extraction method (IT 1-2-3™ VIBE Sample Purification Kit) followed by testing with the JBAIDS Tularemia Detection Kit. JBAIDS operators were blinded to the analyte content of the samples. The results obtained with the Platinum Path processed samples were compared to the results obtained with the VIBE processed samples.

The JBAIDS result for a sample purified using from the VIBE kit was considered the correct result. The mean Cp values decrease as the analyte concentrations increase. There were two false positive Tularemia results obtained during testing. One unspiked sample had a false positive test result when purified with the Platinum Path kit. The other false positive test result was for a Platinum Path purified sample spiked at the 1 × LoD level. This sample tested negative when processed with the VIBE kit (for which results are considered “truth”), so the final interpretation for the sample was “negative” despite the sample actually having been spiked with *F. tularensis* at the 1 × LoD level. When a sample is spiked at the 1 × LoD level, ≥ 95% of results are expected to be positive. Occasional negative results are therefore not unexpected (approximately 1 out of 20), with the consequence in this case of a false positive comparative result for a specimen spiked at the 1 × LoD level. The table below provides the JBAIDS Tularemia test results and Cp values stratified by *F. tularensis* spike level.

**Cp Analysis of JBAIDS Tularemia Detection Kit Testing of Surrogate Spiked Sputum Samples Processed with the IT 1-2-3 Platinum Path and VIBE Sample Purification Kits**

<i>F. tularensis</i> Spike Level	Sample Purification Kit					
	Platinum Path			VIBE		
	JBAIDS Positive/ Total	Mean Cp	SD	JBAIDS Positive / Total	Mean Cp	SD
No spike	1/50	42.5 <sup>a</sup>	-	0/50	-	-
1 × LoD	20/20	34.54	2.01	19/20	36.90 <sup>b</sup>	2.32
5 × LoD	10/10	32.38	2.43	10/10	32.68	2.15
10 × LoD	10/10	31.09	1.91	10/10	32.10	2.83
100 × LoD	5/5	26.45	1.64	5/5	27.77	2.37
1,000 × LoD	5/5	25.32	1.81	5/5	27.16	1.78
<b>≥ LoD</b>	<b>50/50</b>	-	-	<b>49/50</b>	-	-

<sup>a</sup> False positive result for an unspiked sample purified with Platinum Path.

<sup>b</sup> Does not include a Cp for the VIBE-purified sample spiked at the 1 × LoD level with a negative result.

The final Tularemia result for samples purified using the Platinum Path kit had a positive percent agreement (PPA) of 100% as compared to samples purified using VIBE (49/49; 95% CI = 92.8-100%). The final JBAIDS Tularemia result for samples purified using Platinum Path was negative for 49 out of 51 samples that were negative when purified using VIBE. This represents a negative percent agreement (NPA) of 96.1% (49/51; 95% CI = 86.5-99.5%). The IT 1-2-3 VIBE and Platinum Path Sample Purification Kits performed equivalently with respect to detection. The table below presents the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for the surrogate sputum specimen testing.

**JBAIDS Tularemia Detection Kit Performance on Spiked Sputum Samples Processed with the IT 1-2-3 Platinum Path and VIBE Sample Purification Kits**

Positive Agreement				Negative Agreement			
VIBE + Platinum Path +	VIBE + Platinum Path -	PPA	95% CI	VIBE - Platinum Path -	VIBE - Platinum Path +	NPA	95% CI <sup>a</sup>
49	0	100% (49/49)	92.8- 100%	49	2 <sup>a</sup>	96.1% (49/51)	86.5- 99.5%

<sup>a</sup> False positive results obtained for one unspiked sample processed with Platinum Path, and for one sample spiked at the 1 × LoD level (positive result after Platinum Path processing, but negative after VIBE processing).

4. Clinical cut-off:

Not applicable

**N. Instrument Name:**

JBAIDS Instrument

**O. System Descriptions:**

1. Modes of Operation:

See Section, I. Device Description

2. Software:

There was no change made in the software. Refer to previously FDA-cleared 510(k) Premarket Notifications K072547.

3. Specimen Identification:

There was no change made in the specimen identification process. Refer to previously FDA-cleared 510(k) Premarket Notifications K072547.

4. Specimen Sampling and Handling:

There was no change made in the specimen sampling and handling process. Refer to previously FDA-cleared 510(k) Premarket Notifications K072547.

5. Calibration:

There was no change made in calibrators. Refer to previously FDA-cleared 510(k) Premarket Notifications K072547.

6. Quality Control:

There was no change made in the quality controls. Refer to previously FDA-cleared 510(k) Premarket Notifications K072547.

**P. ~~Other Supportive Instrument Performance Characteristics Data Not Covered in the~~ “Performance Characteristics” Section above:**

Not Applicable

**Q. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**R. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.