510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K131729

B. Purpose of Submission:

The purpose of this 510(k) submission is to add the IT *1-2-3* Platinum Path Sample Purification Kit as an accessory for use with the previously cleared JBAIDS Plague Detection Kit.

C. Measurand:

The two target assays in the JBAIDS Plague Detection kit each detect a unique *Y. pestis* DNA sequence: Target 1 detects a sequence on the *pesticin* (*pst*) gene found on the 9.5-kb pPCP1 plasmid (also known as pPla, pPst, and pYP) and Target 2 detects a sequence on the capsule-like antigen fraction 1 (*caf1*) gene found on the 96-kb plasmid pMT1 (also known as pFra, pTox, pYT). The exact gene targets and sequences of the primers and probes used in these assays are considered classified information and therefore were not described in the submission.

D. Type of Test:

The JBAIDS Plague Detection Kit uses real-time PCR with hydrolysis probes to detect *Y. pestis* DNA.

E. Applicant:

BioFire Diagnostics, Inc.

F. Proprietary and Established Names:

JBAIDS Plague Detection Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR section 866.3945, In vitro diagnostic device for *Yersinia* spp. detection

2. Classification:

   Class II

3. Product code:

   OIH

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Joint Biological Agent Identification and Diagnostic System (JBAIDS) Plague 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 *Yersinia pestis*. The kit 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 septic or pneumonic plague. In addition, positive blood cultures and colonies may be tested. The JBAIDS Plague Target 2 assay is used as a supplementary test only after a positive result with the Target 1 Assay.

   The JBAIDS Plague Target 1 and Target 2 assays are run on the JBAIDS instrument using the Diagnostic Wizard. Results are for the presumptive identification of *Y. pestis* in conjunction with culture and other laboratory tests. The definitive identification of *Y. pestis* from colony growth, liquid blood culture growth, or from blood 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 plague must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the identification of *Y. pestis* from cultures or directly from whole blood or sputum specimens.

   The JBAIDS Plague Detection Kit is intended for use by trained clinical laboratory personnel who have received specific training on the use of the JBAIDS Plague Detection Kit. The level of *Y. pestis* that would be present in blood or sputum from individuals with early systemic infection is unknown. Due to the difficulty in obtaining clinical specimens, these assays were not evaluated with blood or sputum from individuals with septic or pneumonic plague.

2. Indication(s) for use:

   Not Applicable

3. Special conditions for use statement(s):

   The JBAIDS Plague Detection Kit is intended for use by trained clinical laboratory personnel who have received specific training on the use of the JBAIDS Plague Detection Kit. The level of *Y. pestis* that would be present in blood or sputum from individuals with early systemic infection is unknown. Due to the difficulty in obtaining clinical specimens, these assays were not evaluated with blood or sputum from individuals with, septic, or pneumonic plague.

4. Special instrument requirements:

   JBAIDS instrument

I. Device Description:

The Joint Biological Agent Identification and Diagnostic System (JBAIDS) Plague Detection System is a fully integrated IVD system composed of the portable JBAIDS instrument, laptop computer and software and the JBAIDS Plague Detection Kit with two different freeze-dried PCR assays for detection of *Yersinia pestis* DNA. The system has been validated using four different sample preparation kits for isolating DNA from whole blood (IT *1-2-3* Platinum Path and QFLOW^{DNA} Sample Purification Kits), sputum (IT *1-2-3* Platinum Path and IT *1-2-3* VIBE), positive blood cultures (IT *1-2-3* SWIPE Sample Purification Kit), and plate cultures (IT *1-2-3* Platinum Path and SWIPE Sample Purification Kits). Use of the JBAIDS DNA Extraction Control Kit is also recommended.

Prior to testing, specimens are processed using BioFire Diagnostic's IT *1-2-3* Sample Purification Kits. The resulting purified sample is added to Target 1 Unknown and Target 1 Inhibition Control vials, along with reconstitution buffer. Target 1 Positive Control and Negative Control vials are prepared using reconstitution buffer and water. When *Y. pestis* DNA is present, a fragment of *Y. pestis* DNA is amplified. The amplicon is detected by fluorescence using a specific hydrolysis probe. Each probe is labeled on one end with a fluorescent reporter moiety (6-carboxyfluorescein (6-FAM)) and elsewhere with a quencher moiety (carboxy tetramethylrhodamine (TAMRA)). When the probe is intact, the quencher absorbs the light emitted by the reporter moiety. During PCR, the probe hybridizes to the target sequence before the exonuclease activity of Taq polymerase hydrolyzes the probe, separating the fluorophore from the quencher and permitting detection of the fluorescent signal generated by the reporter. The fluorescent signal increases as additional templates are amplified and more probes are hydrolyzed.

JBAIDS Software analyzes the fluorescence amplification curves and reports results as positive, negative, uncertain or inhibited. A failure of the Positive or Negative Control will result in the entire run being called invalid. Retesting is required to resolve uncertain, invalid or inhibited results. The Target 2 assay is used as a supplementary test only after a positive result is obtained with the Target 1 assay.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   JBAIDS Plague Detection Kit

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

   K072631

3. Comparison with predicate:

Similarities		
Element	**New Device: JBAIDS Plague Detection Kit**	**Predicate: JBAIDS Plague Detection Kit (K072631)**
Intended Use	Identification of Plague infection through the detection of two DNA sequence targets unique to *Yersinia pestis*. Results are used in conjunction with clinical information, culture, and other laboratory tests as an aid in the diagnosis of systemic Plague infection in individuals suspected of having the disease.	Same
Technology	Real-time PCR using hydrolysis probes	Same
Organism Detected	Qualitative *in vitro* detection of *Yersinia pestis* DNA	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 septic or pneumonic plague, blood culture (grown in soybean-casein digest broth) or bacterial culture (grown on blood agar)	Same
Platform	JBAIDS Instrument	Same
Time Required for Analysis of Specimen	Less than 3 hours	Same
DNA Extraction Method	Blood culture purified with IT *1-2-3*™ SWIPE Sample Purification Kit (or validated equivalent).	Same

Differences		
Element	**New Device: JBAIDS Plague Detection Kit**	**Predicate: JBAIDS Plague Detection Kit (K072631)**
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).
	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 Referenced (if applicable):

FDA 21 CFR 820: QSR, October 7, 1996

21CFR809.10, In Vitro Diagnostic Products for Human Use, Subpart B – Labeling, April 2012.

"Molecular Diagnostic Methods for Infectious Diseases," CLSI Approved Guideline, MM3-A2 (February 2006).

"Evaluation of Precision Performance of Quantitative Measurements Methods; Approved Guidance-Second Edition", CLSI Approved Guidance EP5-A2 (August 2004).

"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: K072631

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

Reproducibility

This study was designed to establish test result reproducibility using the JBAIDS Plague Detection Kit in conjunction with the IT *1-2-3* Platinum Path Sample Purification Kit. During the study each purified sample was tested with both target assays. Qualitative Plague assay results were evaluated for reproducibility by testing replicate whole blood and sputum samples over several runs, on several days, at multiple test sites.

Contrived specimens were prepared by spiking freshly collected pooled human whole blood or frozen pooled sputum with inactivated *Y. pestis*. Spike levels consisted of the following:

- true negative samples
- low positive samples, spiked at limit of detection (LoD*)
- medium positive samples, spiked at 5×LoD*

  * In the live organism LoD study, the Cp values obtained when testing unpurified dilutions of the inactivated organism stock and live organism stock (both concentrations reported in CFU/mL) indicated that the inactivated organism stock (i.e. the lot to be used in this study) contains less of the gene target than the live organism stock. To account for these differences, the inactivated organism was used at a concentration that yielded a PCR performance similar to the live organism LoD, and the concentrations are reported in 'live equivalence'. For the whole blood matrix the equivalent inactivated organism concentrations are 25x higher (in CFU/ml) than the live organism LoD and the sputum matrix equivalent inactivated organism concentrations are 5x higher (in CFU/ml) than the live organism LoD.

A panel of twelve human whole blood specimens and a panel of nine human sputum specimens spiked with inactivated *Y. pestis* at the two concentrations noted above were tested twice each day for four days (whole blood) or five days (sputum) at each of three testing sites. There were 96 total whole blood sample replicates and 90 total sputum sample replicates tested at each replicate level. On each testing day, two users at each site tested one aliquot of each of the specimens in the panel. A minimum of two JBAIDS instruments were used at each site. At least two lots of JBAIDS Plague reagents were used over the course of the 4 days of whole blood sample testing at each site (three lots were used at Site 3), and at least three lots of JBAIDS Plague reagents were used over the course of the 5 days of sputum sample testing at each site (four lots at Site 3). One set of DNA Extraction Controls (EC), one positive EC sample and one negative EC sample, was prepared and purified during each round of sample purification.

Run control (NC and PC) results

Negative Control (NC) and Positive Control (PC) reactions were included in every JBAIDS run. These are freeze-dried reagent vials that either omit or include template DNA, respectively, for the target assays. In 124 out of 124 runs and 115 out of 117 runs for Target 1 and Target 2 respectively, the NC reactions were successful. In all Target 1 and Target 2 runs the PCs were successful. The run control results establish acceptable reproducibility with standard deviations of less than one cycle for both target PCs.

Inhibition control (IC) results

A target-specific inhibition control assay (IC) is run with each sample. The IC result is "inhibited" when the associated IC assay is unsuccessful and the plague target assay is not positive. There were 288 processed whole blood samples tested with both the Target 1 and Target 2 assays. For one sample, an initial "inhibited" result for Target 1 resolved as a positive result on retesting. For one sample, initial results were "inhibited" for both the Target 1 and Target 2 assays; the Target 1 assay result was "inhibited" again on retesting, and there was inadequate volume to retest the purified sample for Target 2. Therefore, 0.35% (1/288) of whole blood samples produced final results of "inhibited" for both Target 1 and Target 2 assays.

There were 270 processed sputum samples tested with both the Target 1 and Target 2 assays. Of the eleven samples with initial "inhibited" results for Target 1, one produced a negative result on retesting and nine produced positive results on retesting. Of the five samples with initial "inhibited" results for Target 2, all five produced negative results on retesting.

Extraction control (EC) results

Twenty-five out of 25 whole blood Platinum Path purifications were successful. However, only 27/31 sputum Platinum Path extractions were successful (87%). This unexpectedly low success rate for the DNA ECs in sputum purifications was resolved after this study was completed as summarized in Section 7.2, under Protocol Deviations and Non-Conformities. The EC failures were attributed to dilution errors when preparing the samples. The EC failures were not re-run since there were no false assay results reported during this study.

Assay reproducibility results (whole blood)

When results from all three trial sites were combined, samples spiked at or above the LoD were detected >99% of the time when tested with both assays. True negative samples were not detected >97.9% of the time, with two unresolved inhibited results (neither detected nor undetected) and one non-negative result for the Target 2 assay only. The one non-negative result did

not produce a final false positive result for Plague since both targets must be detected for final positive result.

At analyte levels above the LoD, the JBAIDS Plague Detection system variability, as measured by the coefficient of variation (% CV) of the Cp values, was below 4.5% for all samples spiked at or above the LoD, regardless of test site. Some site-to-site variation in mean Cp values was observed. However, the error in the mean Cp as measured by the standard deviation is overlapping across sites at both analyte levels.

Assay reproducibility results (sputum)

Samples spiked at both the medium positive level (5×LoD) and the low positive level (1×LoD) yielded positive results >98% of the time. Samples that were not spiked with inactivated organism (true negatives) yielded negative results 98.9% of the time for Target 1 and 100% of the time for Target 2. The one uncertain result obtained for a true negative sample was also uncertain on retest of the same sample. A number of the true negative samples initially had inhibited results, which resolved to negative on retesting. There were no observed differences in qualitative reproducibility results of Plague assays between days, users, or between sites testing on at least two instruments.

When results from all three trial sites were combined, detection at or above the LoD level was positive >99% of the time for each assay. True negative samples were not detected 100% of the time for the Target 2 assay and 99% of the time for the Target 1 assay. There were no false positive results, but one of the true negative samples produced an uncertain result for Target 1 only (negative for Target 2). The uncertain result for plague resolved to a negative result when the sample was re-purified and re-tested for both targets as directed in the JBAIDS Plague Detection Kit Instruction Booklet.

The submitted reproducibility data is acceptable and supports the performance claim stated in the product labeling.

b. Linearity/assay reportable range:

Not applicable.

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

There are no reported changes to the stability or expected values therefore no study was recommended during the presubmission for this assay. For information on stability and expected values refer to previously FDA-cleared 510(k) Premarket Notifications K072631.

d. Detection limit:

The purpose of this study was to establish that the JBAIDS Plague Detection Kit used in conjunction with the IT *1-2-3* Platinum Path Sample Purification Kit (Platinum Path) for whole blood and sputum samples meets the previously established LoD of 50 CFU/mL and 670 CFU/mL of *Yersinia pestis*, respectively. The testing was performed in two phases.

Phase one was a direct comparison of the test result obtained with the previously validated purification kit (i.e. QFLOW^{DNA} for whole blood and VIBE for sputum), and the new purification kit (Platinum Path) performed by spiking (whole blood or sputum) samples with *Y. pestis* at 10×, 1×, and 0.1× the previously established LoD (50 CFU/mL for whole blood, 670 CFU/mL for sputum). The samples were then split and purified in parallel using both purification methods followed by testing with the JBAIDS Plague Detection Kit Target 2 assay. The Target 2 assay was selected as the representative assay to compare the two sample purification kits. If the dilution series demonstrated similar assay sensitivity for both purification methods, then the second phase of confirmation testing was initiated.

Phase two was a confirmation of the whole blood and sputum LoDs performed by purifying 20 individually prepared whole blood samples spiked at 50 cfu/mL and 20 individually prepared sputum samples spiked at 670 cfu/mL with *Y. pestis*, followed by purification only with the Platinum Path kit. Confirmation required at least 95% (19/20) positive detection of samples spiked at the LoD with both of the Target 1 and Target 2 assays.

Initial Plague Target 2 test results comparing the previously validated purification kits and the new purification kit for whole blood and sputum samples are summarized in Table 1. At the 10× LoD level, 100% of spiked whole blood and sputum samples were detected, regardless of the purification method used. At the 1× LoD level, most samples were detected (75-100% detection) and below the LoD level, few samples were detected (25%). One exception was the of the Platinum Path processed sputum samples, which were 100% detected at the 0.1× LoD level and required additional testing at lower template levels (0.01× LoD) to observe reduced detection (i.e. 0%).

In the initial direct comparison testing, qualitative Target 2 detection of *Y. pestis* was similar for whole blood samples processed with the QFLOW^{DNA} kit or the Platinum Path kit.

Table 1. Range finding data from Phase One LoD study

***Y. pestis* Spiked Concentraion (x Lod)**	**Whole Blood**		**Sputum**	
	Platinum Path	**QFLOW^{DNA}**	**Platinum Path**	**QFLOW^{DNA}**
	Detected/Total (% Detected)	**Detected/Total (% Detected)**	**Detected/Total (% Detected)**	**Detected/Total (% Detected)**

Table 1. Range finding data from Phase One LoD study				
***Y. pestis* Spiked Concentraion (x Lod)**	**Whole Blood**		**Sputum**	
	Platinum Path	**QFLOW^{DNA}**	**Platinum Path**	**QFLOW^{DNA}**
	Detected/Total (% Detected)	**Detected/Total (% Detected)**	**Detected/Total (% Detected)**	**Detected/Total (% Detected)**
10×	4/4 (100%)	4/4 (100%)	4/4 (100%)	4/4 (100%)
1×	3/4 (75%)	4/4 (100%)	8/8 (100%)	7/8 (100%)
0.1×	1/4 (25%)	1/4 (25%)	8/8 (100%)	2/8 (25%)
0.01×	NA	NA	0/8 (0%)	0/8 (0%)

In confirmation testing, twenty out of 20/20 (100%) whole blood samples spiked at 50 CFU/mL and purified with the Platinum Path kit were positive for the Plague Target 1 assay and 19/20 (95%) were positive for the Target 2 assay. For both the Plague Target 1 and Target 2 assays, 20/20 (100%) sputum samples spiked at 670 CFU/mL and purified with the Platinum Path kit were positive.

The LoD study data is acceptable and supports the performance claims of 50 CFU/mL for whole blood specimens and 670 CFU/mL for sputum specimens.

e. Analytical reactivity/specificity:

No changes to the assay primers or probes are reported therefore no study was recommended during the presubmission for this assay. Analytical reactivity/specificity data for this assay can be found in K072631.

f. Assay cut-off:

No changes in the assay cut-off are reported. For assay cut-off data, refer to previously FDA-cleared 510(k) Premarket Notifications K072631.

g. Interfering species

There are no reported changes to the assay primers or probes therefore no interfering species study was recommended during the presubmission for this assay. Interfering species data for this assay can be found in K072631.

h. Direct from culture testing

This study evaluated the ability of the JBAIDS Plague Detection Kit to identify and detect *Y. pestis* 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 previously validated SWIPE protocol that previously cleared for use with the JBAIDS Plague Detection Kit (K072631),

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 *Y. pestis* CO92 strain colonies to demonstrate that the test system detects *Y. pestis*. Colonies of *B. anthracis* and *F. tularensis* 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 *Y. pestis* colonies and 10 individual non- *Y. pestis* colonies (five from the *B. anthracis* cultures and five from the *F. tularensis* cultures) were processed using the Platinum Path "Direct Testing of Bacterial Cultures Protocol" and tested with the JBAIDS Plague Detection Kit.

There were no unexpected results for the testing of ten *Y. pestis* colonies and ten non- *Y. pestis* colonies. All samples containing *Y. pestis* yielded positive results, and no *Y. pestis* was detected in any of the negative control samples (of *B. anthracis* or *F. tularensis*). The Target 1 and Target 2 Cp values for the *Y. pestis* colonies were fifteen cycles earlier than the Cp values for whole blood samples spiked at the LoD and purified by Platinum Path.

2. Comparison studies:

   a. Method comparison with predicate device:

      Not applicable

   b. Matrix comparison:

      Not applicable

3. Clinical studies:

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 Plague Detection Kit. Because clinical specimens from patients infected with *Yersinia pestis* (plague) are not available, spiked samples were used in the clinical evaluations. The first evaluation used prospectively collected whole blood specimens collected in sodium citrate and obtained from patients with febrile illness. The second evaluation used residual frozen sputum specimens that were spiked with inactivated *Y. pestis*. In both evaluations, the spiked samples were purified in parallel using the new and old extraction methods, and then tested with the JBAIDS Plague Detection Kit.

Whole blood specimen testing

Subjects meeting the following criteria were invited to enroll in the study at the three study sites (after informed consent was acquired):

Inclusion Criteria

- Subject experienced a fever of at least 100˚F within the previous 24 hours (recorded or self-reported).
- Subject was an adult.

Exclusion Criteria

- Adult volunteers who were incapacitated or otherwise unable to provide informed consent were excluded from the study.
- Individuals under the age of 18 were excluded from the study.

Whole blood specimens were collected at three sites that were selected for the quality of their personnel and their access to the desired subject populations. The study locations were representative of the intended use setting (military hospitals and field clinics), and specimen collection was performed by trained study personnel.

De-identified blood specimens were collected in two 5 ml sodium citrate tubes and stored at 2-8°C. Blood specimens were shipped on ice to BioFire Diagnostics labs twice a week. Upon arrival at the Firm, samples were assigned a new study code number and then spiked with inactivated *Y. pestis* (CO92 strain) according to a randomized spiking plan. A total of one hundred (100) samples were spiked as shown in Table 2.

Table 2. Human whole blood sample spike levels and number of replicates

***Y. pestis* spike level**	**Live Organism in CFU/ml (Inactivated Organism Equivalent)***	**Number of Sample Replicates Processed with Each Sample Purification Kit**
No Spike	-	50
1× LoD	50 (1250)	20
5× LoD	250 (6250)	10
10× LoD	500 (12,500)	10
100× LoD	5,000 (125,000)	5
1,000× LoD	50,000 (1,250,000)	5
	Total	100

* As noted in Section M.1.a (Assay Reproducibility) a change in reactivity of inactivated organisms compared with live organisms was observed by the Firm. This change in reactivity required a higher concentration of inactivated

organism in CFU/ml to achieve the same cp. Whole blood specimens were spiked with inactivated organism at a 25 fold higher concentration (in terms of CFU/ml) in order to achieve the same Cp value as live organisms.

All blood samples were tested within seven days of blood collection. The operator purifying and testing the samples were blinded to the spike level and the expected test result. Two aliquots from each prepared sample were purified, one with the Platinum Path kit and the other with the QFLOW^{DNA} kit. The purified samples were then tested with the JBAIDS Plague Detection Kit.

The results for the Platinum Path processed samples were compared to the results of the QFLOW^{DNA} processed samples, which were accepted as the correct results (no further compactor method testing was performed.)

The mean Cp values decrease as the analyte concentrations increase as shown in Table 3. Regardless of the analyte concentration, amplification of Platinum Path purified samples is consistently ~1.5 to 2.0 cycles later when compared to QFLOW^{DNA} purified samples for both target assays. However, qualitative results for samples purified by the two purification kits are similar. *Y. pestis* was detected by both target assays in 100% of the specimens spiked at the 1×LoD level and purified with Platinum Path. For the same specimens purified with QFLOW, *Y. pestis* was detected by both target assays in 19/20 (95%) of the spiked specimens. A single discordant result was obtained for a sample that was spiked at the LoD. When a sample is spiked at the 1×LoD level, ≥ 95% of results are expected to be positive. Occasional negative results are therefore expected (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. At all other analyte concentrations qualitative performance was identical regardless of the purification method used.

Table 3. Cp analysis of JBAIDS Plague Detection Kit testing of spiked whole blood samples processed with the IT 1-2-3 Platinum Path and QFLOW sample purification kits

***Y. pestis* Spike Level**	**Plague Target Assay**	**Sample Purification Kit**					
		Platinum Path			**QFLOW^{DNA}**		
		JABAIDS Positive/Total	**Mean Cp**	**SD**	**JABAIDS Positive/Total**	**Mean Cp**	**SD**
No Spike	**Target 1**	0/50			0/50		
	Target 2	0/50			0/50		
1X LoD	**Target 1**	20/20	30.07	0.82	19/20	28.61	0.78
	Target 2	20/20	32.72	0.80	19/20	31.00	0.70
5X LoD	**Target 1**	10/10	27.92	0.74	10/10	26.40	1.04
	Target 2	10/10	30.90	0.76	10/10	28.08	0.85
10X LoD	**Target 1**	10/10	27.05	0.85	10/10	25.36	0.96
	Target 2	10/10	30.24	0.89	10/10	29.2	0.90
100X	**Target 1**	5/5	23.33	0.76	5/5	21.63	0.48

Table 3. Cp analysis of JBAIDS Plague Detection Kit testing of spiked whole blood samples processed with the IT 1-2-3 Platinum Path and QFLOW sample purification kits

Y. pestis Spike Level	Plague Target Assay	Sample Purification Kit					
		Platinum Path			QFLOW^{DNA}		
		JABAIDS Positive/Total	Mean Cp	SD	JABAIDS Positive/Total	Mean Cp	SD
LoD	**Target 2**	5/5	26.64	0.97	5/5	24.60	0.54
1,000X LoD	**Target 1**	5/5	20.47	0.82	5/5	18.92	1.39
	Target 2	5/5	23.83	0.69	5/5	21.98	1.34

Relative performance of both purification kits is shown in Table 4, where the result for a sample purified with the QFLOW^{DNA} kit was accepted as the correct result. The final Plague interpretation for samples purified using the Platinum Path kit had a positive percent agreement (PPA) of 100% as compared to samples purified using QFLOW^{DNA} (49/49; 95% CI = 92.7-100%). The final JBAIDS Plague interpretation for samples purified using Platinum Path was negative for 50 out of 51 samples that were negative when purified using QFLOW^{DNA}. This represents a negative percent agreement (NPA) of 98% (50/51; 95% CI = 89.6-100%). Overall percent agreement between the two purification kits is 99% (99/100; 95% CI = 94.6-100%).

Table 4. JBAIDS Plague Detection Kit Performance on Spiked Whole Blood Samples Processed with the IT 1-2-3 Platinum Path and QFLOW Sample Purification Kits

		QFLOW Sample preparation method	
		Positive	**Negative**
Platinum Path sample preparation method	**Positive**	49	1
	Negative	0	50
		Two sided 95% confidence interval	
Positive percent agreement (PPA)	100 % (49/49)	92.7% - 100%	
Negative percent agreement (NPA)	98% (50/51)	89.6% - 99.9%	

Sputum specimen testing

One hundred (100) surrogate samples were prepared using de-identified frozen residual sputum specimens spiked with inactivated *Y. pestis* at levels shown in Table 5. Fifty (50) of the specimens were spiked at concentrations near and above the system LoD, while the remaining 50 specimens were not spiked with *Y. pestis.*

Table 5. Sputum sample spike levels and number of replicates

***Y. pestis* spike level**	**Live Organism in CFU/ml (Inactivated Organism Equivalent)***	**Number of Sample Replicates Processed with Each Sample Purification Kit**
No Spike	-	50
1X LoD	670 (3350)	20
5X LoD	3,350 (16,750)	10
10X LoD	6,700 (33,500)	10
100X LoD	67,000 (335,000)	5
1,000 X LoD	670,000 (3,350,000)	5
	Total	100

* As noted in Section M.1.a (Assay Reproducibility) a change in reactivity of inactivated organisms compared with live organisms was observed by the Firm. This change in reactivity required a higher concentration of inactivated organism in CFU/ml to achieve the same Cp. Sputum specimens were spiked with inactivated organism at a 5 fold higher concentration (in terms of CFU/ml) in order to achieve the same Cp value as live organisms.

Each specimen was prepared in duplicate and one was purified using the Platinum Path kit and the other purified with the VIBE kit. The purified samples were then tested with the JBAIDS Plague Detection Kit. The results for the Platinum Path processed samples were compared to the results of the VIBE processed samples, which were accepted as the correct results.

Valid JBAIDS Plague detection results were obtained for 100 contrived sputum samples purified by both the Platinum Path and VIBE Sample Purification Kits. Table 6 provides the test results and Cp values for the JBAIDS Plague Target 1and Target 2 assays stratified by *Y. pestis* spike level. As expected, the mean Cp values generally increased as the analyte concentration decreased. Samples processed with the Platinum Path kit and samples processed with the VIBE kit for either target assay showed 100% agreement for samples spiked at ≥ the LoD. There was one Platinum Path purified sputum sample that was not spiked with *Y. pestis* that gave a false positive result for both Plague target assays.

Table 6. Cp Analysis of JBAIDS Plague Detection Kit Testing of contrived spiked sputum samples processed with the IT 1-2-3 Platinum Path and VIBE Sample Purification Kits

***Y. pestis* Spike Level**	**Plague Target Assay**	**Sample Purification Kit**					
		Platinum Path			**VIBE**		
		JABAIDS Positive/Total	**Mean Cp**	**SD**	**JABAIDS Positive/Total**	**Mean Cp**	**SD**
No Spike	**Target 1**	1/50	31.51		50/50		

Table 6. Cp Analysis of JBAIDS Plague Detection Kit Testing of contrived spiked sputum samples processed with the IT 1-2-3 Platinum Path and VIBE Sample Purification Kits

Y. pestis Spike Level	Plague Target Assay	Sample Purification Kit					
		Platinum Path			VIBE		
		JABAIDS Positive/Total	Mean Cp	SD	JABAIDS Positive/Total	Mean Cp	SD
	Target 2	1/50	32.90		50/50		
1X LoD	**Target 1**	20/20	29.30	1.71	20/20	28.74	2.00
	Target 2	20/20	32.23	1.57	20/20	31.41	1.93
5X LoD	**Target 1**	10/10	26.02	1.63	10/10	26.62	2.53
	Target 2	10/10	28.80	1.65	10/10	29.31	2.15
10X LoD	**Target 1**	10/10	26.65	3.28	10/10	27.68	2.22
	Target 2	10/10	29.69	3.4	10/10	30.43	1.82
100X LoD	**Target 1**	5/5	23.41	2.48	5/5	22.35	2.61
	Target 2	5/5	26.23	2.44	5/5	25.38	2.49
1,000X LoD	**Target 1**	5/5	19.78	2.39	5/5	20.82	2.08
	Target 2	5/5	22.62	2.57	5/5	23.71	2.17

Relative performance of both purification kits is shown in Table 7, where the result for a sample purified with the VIBE kit was accepted as the correct result. The final Plague interpretation for samples purified using the Platinum Path kit had a PPA of 100% as compared to samples purified using VIBE (50/50; 95% CI = 92.9-100%). The final JBAIDS Plague interpretation for samples purified using Platinum Path was negative for 49 out of 50 samples that were negative when purified using VIBE. This represents a NPA of 98% (49/50; 95% CI = 89.4-100%).

Table 7. JBAIDS Plague Detection Kit Performance on Spiked Sputum Samples Processed with the IT 1-2-3 Platinum Path and VIBE Sample Purification Kits

		VIBE Sample preparation method	
		Positive	**Negative**
Platinum Path sample preparation method	**Positive**	50	1
	Negative	0	49
		Two sided 95% confidence interval	
Positive percent agreement (PPA)	100 % (50/50)	92.9% - 100%	
Negative percent agreement (NPA)	98% (49/50)	89.4% - 100%	

4. Clinical cut-off**:**

   Not Applicable.

5. Expected values/Reference range:

   Not Applicable.

N. Instrument Names:

Joint Biological Agent Identification and Diagnostic System

O. System Descriptions:

1. Modes of Operation:

   No changes in the mode of operation were reported. For information on the modes of operation refer to previously FDA-cleared 510(k) Premarket Notifications K072631.

2. Software:

   No changes in the software were reported. For software information refer to previously FDA-cleared 510(k) Premarket Notifications K072631.

3. Specimen Identification:

   No changes in the specimen identification were reported. For specimen identification information refer to previously FDA-cleared 510(k) Premarket Notifications K072631.

4. Specimen Sampling and Handling:

   No changes in the specimen sampling and handling were reported. For specimen sampling and handling identification information refer to previously FDA-cleared 510(k) Premarket Notifications K072631.

5. Calibration:

   No changes in calibration were reported. For specimen calibration information refer to previously FDA-cleared 510(k) Premarket Notifications K072631.

6. Quality Control:

   No changes in quality control were reported. For quality control information refer to previously FDA-cleared 510(k) Premarket Notifications K072631.

P. Other Supportive Instrument Performance Characteristics Data Not Covered in the "Performance Characteristics" Section above:

None

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.