510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number: K072631

B. Purpose for Submission: New test system

C. Measurand: *Yersinia pestis* unique DNA sequences

D. Type of Test: Real-time fluorescent PCR assay for *in vitro* diagnostic detection of target DNA sequence of *Yersinia pestis*; qualitative result

E. Applicant: Idaho Technology, Inc.

F. Proprietary and Established Names: Joint Biological Agent Identification and Diagnostic System (JBAIDS) Plague Detection System; Real-time PCR amplification and detection system for targeted *Y. pestis* DNA sequences.

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OIH, assay, Nucleic Acid Amplification, *Yersinia pestis*	Class II (by Panel Recommendation)	Unclassified	Microbiology (83)

H. Intended Use:

1) Intended use: 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

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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:

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.

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.

3) Special instrument requirements:

Not applicable.

I.   Device Description:

The JBAIDS Plague Detection Kit is an integrated *in vitro* diagnostic (IVD) system composed of the JBAIDS instrument with a laptop computer, software and two different freeze-dried sets of reagents(Target 1 and 2 assays in one kit) for performing real-time PCR in glass capillaries. The Kit contains two real-time PCR assays.

Real-time PCR is accomplished with the use of a hydrolysis probe, which detects a specific sequence within the region amplified by the assay primers. The probe is labeled on one end with a fluorescent reporter molecule (6-FAM) and on the other end with a quencher (TAMRA). When the probe is intact, the quencher absorbs the light emitted by the reporter molecule. During PCR, the intact probe hybridizes to the amplicon, and the exonuclease activity of Taq polymerase separates the fluorophore from the quencher, generating a fluorescent signal that is detected by the instrument. The fluorescent signal increases as additional template is amplified and more probe is hydrolyzed. The primers and probes were selected based upon two basic criteria: 1) they detect all sequenced virulent isolates of *Y. pestis;* and 2) they do not detect sequences in other organisms.

J. Substantial Equivalence Information:

The JBAIDS Plague Detection Kit is the first-of-kind, there are no legally marketed predicate device. At the FDA's Microbiology Devices Advisory Panel on Mar. 7, 2002 the panel determined that these devices would be Class II and could be compared to preamendment DFA devices.

K. Standard/Guidance Document Referenced (if applicable):

- Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens, FDA Guidance Document (Draft: Dec. 8, 2005)
- User Protocol for Valuation of Qualitative Test Performance, Clinical Laboratory Standards Institute(CLSI) Approved Guidelines, EP12-A (Aug. 2002).
- Molecular Diagnostic Methods for Infectious Diseases, CLSI Approved Guidelines, MM3-A (Dec. 1995).
- Interference Testing in Clinical Chemistry, CLSI Approved Guideline EP7-A (Dec. 2002)

L. Test Principle:

The JBAIDS Plague Detection Kit is a real-time PCR with hydrolysis probes to detect *Y. pestis* DNA in patient whole blood and sputum samples as well as positive blood cultures and colonies. After extracting DNA from the specimens, real-time PCR is performed into the capillaries. The real-time PCR is accomplished with the use of hydrolysis probe that detects a specific sequence within the region amplified by the assay primers. The probe is labeled on one end with a fluorescent reporter molecule (6-FAM) and on the other end with a quencher (TAMRA). When the probe is intact, the quencher absorbs the light emitted by the reporter molecule. During PCR, the intact probe hybridizes to the amplicon, and the exonuclease activity of Taq polymerase separates the fluorophore from the quencher, generating a fluorescent signal that is detected by the instrument. The fluorescent signal increases as additional template is amplified and more probe is hydrolyzed. Temperature and fluorescence data are displayed in real-time. Each JBAIDS test is analyzed and assigned a final result by the Detector module of the JBAIDS software. Possible final results are positive, negative, inhibited, uncertain, or invalid. To assign a final test result, Detector first analyzes the data from each capillary independently before analyzing the sample duplicates together. Finally, the software assigns a final result, or a combined call, based on the results of the sample and all of its controls.

The characteristics of the amplification curves from the positive control (PC), negative control (NC), inhibition control (IC), and from each unknown sample are automatically analyzed by the JBAIDS Software. Results are reported as Positive, Negative, Inhibited, or Uncertain. When results cannot be determined as positive or negative and are equivocal, the instrument readout displays 'Uncertain'. When PCs or NCs are unacceptable, the test results for all samples in the JBAIDS run are considered invalid and must be repeated.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility: Reproducibility study was performed at three sites on three different days. Sample panels were prepared by spiking either whole blood, sputum, or simulated blood culture specimens with inactivated *Y. pestis* at low (near LOD),

medium, or high level. Negative sample panels were not spiked for each matrix. Overall reproducibility for all three matrices ranges from 94 – 98 % except sputum for target 2 (84.9 %). This data is acceptable for devices of this type. See table below.

Matrix	Target 1				Target 2			
	Site 1 Overall	Site 2 Overall	Site 3 Overall	Overall for all sites	Site 1 Overall	Site 2 Overall	Site 3 Overall	Overall for all sites
Whole Blood	42/42	39/42	42/42	123/126	40/42	42/42	42/42	124/126
	100%	92.90%	100%	97.60%	95.20%	100%	100%	98.40%
Sputum	42/42	37/42	42/42	121/126	36/42	38/42	33/42	107/126
	100%	88.10%	100%	96.00%	85,70%	90.50%	78.60%	84.90%
Blood Culture	18/18	18/18	15/18	51/54	18/18	18/18	17/18	53/54
	100%	100%	83.30%	94.40%	100%	100%	94.40%	98.10%

Between-Site Reproducibility Results for Whole Blood, Sputum, and Blood Culture.

b. *Linearity/assay reportable range:*

   Not applicable.

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

   Positive, Negative and Inhibition controls were included in each run and gave expected results. If not, the runs failed or were called invalid and the run needed to be repeated.

d. *Detection limit:*

   Initially, the Limit of detection (LOD) was estimated by spiking whole blood or sputum specimens with a serial dilution of live *Y. pestis* CO92. Whole blood specimens collected in 3.2% sodium citrate were spiked with 6 to 100 CFU/mL of live *Y. pestis*. Sputum specimens were spiked with 330 to 1000 CFU/mL. Each specimen was purified using the appropriate sample purification kit, IT 1-2-3 QFLOW[dna] for whole blood and IT 1-2-3 VIBE for sputum specimens and the purified samples were tested with the *Y. pestis* assay following standard procedures. Based on the titration results, the LOD was estimated to be 50 CFU/mL for whole blood and 670 CFU/mL for sputum. Reactions were evaluated using the JBAIDS test result, the Cp, and a visual examination of the amplification curves.

   To confirm these LOD levels, 59 whole blood specimens were spiked with 50 CFU/mL and 59 sputum specimens were spiked with 670 CFU/mL live *Y. pestis*

CO92. The specimens were processed and tested, and the frequency of positive results was determined. All 59 spiked whole blood specimens were successfully detected by the JBAIDS Plague Detection Kit, indicating that whole blood specimens containing organism concentrations as low as 50 CFU/mL can be detected with 100% success (95% CI, 95%-100%). For sputum, all 59 specimens were detected with the Target 1 and 56 (94.9%) were detected with the Target 2 assay, indicating that sputum specimens containing *Y. pestis* at 670 CFU/mL can be detected with 100% success (95% CI, 95%-100%) using the Target 1 assay and with 94.9% success (95% CI, 87-100%) with the Target II assay.

e. *Analytical specificity:*

The analytic inclusivity and exclusivity of the JBAIDS Plague Detection Kit was assessed by testing panels of well-characterized organisms. The panels were comprised of: (1) Strains of *Y. pestis* representing the known genetic and geographic distribution of the organism, (2) Closely related organisms, and (3) Unrelated organisms typically found in clinical specimens. *Y. pestis* strains were tested using colonies grown on culture plates as well as with purified DNA at a level equivalent to five times the established nucleic acid LOD of the reference strain, *Y. pestis* CO92. All non-*Y. pestis* organisms were tested using only colonies grown on culture plates.

For all colonies evaluated, the final test result was compared to the expected test result based on microbiological identification methods, and the Cp value for positive samples was evaluated. Organisms in the inclusivity panel were expected to test positive and have Cp values around 20, while organisms in the exclusivity panel were expected to test negative. For the inclusivity panel using purified DNA samples, the test result and Cp value for each sample were evaluated. The LOD for each *Y. pestis* strain was considered equivalent to the reference strain, *Y. pestis* CO92, if the test result was positive and the average Cp value for the strain was within 1.5 cycles of the average Cp value obtained for the reference strain.

Inclusivity strains: When testing colonies, all 22 strains of *Y. pestis* were correctly identified by the JBAIDS Plague Detection Kit. Seventeen of the 22 strains gave positive test results with both the target assays. Three of the strains gave positive results with the Target 1 assay and negative results with the Target 2 assay. Two of the strains gave negative results with the Target 1 assay. The average Cp values obtained for the different strains ranged from 15.45 to 19.33 for the Target 1 assay and 16.11 to 20.11 for the Target 2 assay.

Exclusivity strains: All 24 panel organisms gave the expected negative result with the Target 1 assay, and 23 gave negative results with the Target 2. One strain of *Y. enterocolitica* gave a weak cross reaction when testing with the Target 2 assay. However, the Cp values obtained with this strain were 15 to 20 cycles later than for the reference strain, demonstrating that the cross-reactivity with the Target 2 assay was very weak.

Interfering Substances: The two JBAIDS assays were tested against a panel of potentially interfering substances that could be found in whole blood, sputum,

blood cultures, or colonies and could be introduced during sample purification or reaction set-up. See table below.

The concentration of each substance tested represented a relevant concentration in accordance with CLSI EP7-A *Interference Testing in Clinical Chemistry Approved Guideline*.

All endogenous and exogenous substances typically found in whole blood, sputum, blood cultures, or colonies were spiked into the sample matrix and subjected to sample processing with the appropriate IT 1-2-3 Sample Purification Kit prior to testing with the JBAIDS Tularemia Detection Kit. The sample purification portion of the system is designed to isolate DNA and remove other impurities; therefore, sample processing should eliminate most potentially interfering substances from the purified sample. Because technique-specific substances can be encountered during reaction setup, all such substances were added to the purified samples immediately prior to reaction setup.

Each sample was tested using an Inhibition Control vial and an Unknown vial. The substance was considered to be an interfering substance if the target assay was called negative or inhibited by the JBAIDS Software. A substance was considered to be potentially inhibitory if the Cp of the target assay or the IC assay was delayed by more than three cycles or if the Fmax of either assay was decreased by more than 50% compared to samples with no substance added.

List of Evaluated Potentially Interfering Substances.

Endogenous Substances	Exogenous Substances		
Hemoglobin Albumin Bilirubin Triglycerides Cholesterol (total) Immunoglobulins Glucose	Acetaminophen Amoxicillin Ascorbic acid Aspirin Cefotaxime Chloroquine Ciprofloxacin Doxycycline Erythromycin Gentamicin sulfate	Ibuprofen Naproxen sodium Rifampin Streptomycin Sulfamethoxazole Tetracycline Tobramycin Trimethoprim	Acid-citrate-dextrose Citrate (sodium) EDTA Heparin Sodium polyanethol sulfonate (SPS) Albuterol (Salbutamol) Cromolyn sodium

			Flunisolide (Flovent®) N-acetylcysteine Blood culture media Sheep blood agar media
Solvents Used*	Technique-specific Substances		
Acetone DMSO Ethanol NH$_4$OH	Bleach DNA*Zap* Snap n' Digest	IT 1-2-3™ kit Buffer 1 IT 1-2-3™ kit Buffer 1A IT 1-2-3™ kit Buffer 1B IT 1-2-3™ kit Buffer 1C IT 1-2-3™ kit Buffer 2	QIAGEN Buffer AL QIAGEN Buffer AW1 QIAGEN Buffer AW1 (w/o EtOH) QIAGEN Buffer AW2 QIAGEN Buffer AW2 (w/o EtOH)

*These are solvents used to dissolve potentially interfering substances in preparation for testing.

The following substances were shown to inhibit the JBAIDS Plague detection assays:
- SPS
- Bleach
- DNA*Zap*
- QIAGEN Buffer AL
- QIAGEN Buffer AW1
- QIAGEN Buffer AW1 (no ethanol added)
- ITI Buffer 1A
- ITI Buffer 1B
- ITI Buffer 1C
- ITI Buffer 1

f.   *Assay cut-off:* A data analysis module within assay-specific software applies mathematical modeling of expected amplification curve shapes to each individual capillary. When fixed thresholds with a quadratic formula or crossing point determination are matched, samples are called negative or positive. For undecided samples, an expert system approach is applied that uses filters to assess the fluorescence change around the crossing point.

2. Comparison studies:

a. *Method comparison with predicate device:*

Not applicable.

b. *Matrix comparison:*

The studies were done in sputum, whole blood, cultured isolates and from positive blood culture bottles. Studies showed that there were differences in blood and sputum specimens.

3. Clinical studies:

a.   *Clinical Sensitivity:* This assay was not evaluated with blood or sputum from individuals presenting with signs and symptoms of plague and who subsequently developed septic or pneumonic plague. Users are instructed to establish the clinical sensitivity of this test on prospectively collected clinical specimens.

b.   *Clinical specificity:* A multicenter clinical study was conducted over a 7-month period on 169 patients exhibiting symptoms consistent with systemic *Y. pestis* infection. Patients were selected from those who were hospitalized, exhibited the clinical definition of Systemic Inflammatory Response Syndrome, and had a blood and/or sputum culture ordered. The study subjects ranged in age from 18–94 years (mean 43), and 50.6% were male. Blood samples from 149 subjects were collected and tested, while 45 sputum samples were collected and tested. For 25 subjects, both blood and sputum specimens were tested. The study was conducted at three clinical sites in Maryland, California, and Egypt. The clinical specificity of the JBAIDS Plague Detection System was assessed by comparing the JBAIDS results (Positive/Negative) to the results

obtained by established culture and microbiology follow-up methods. The results are summarized in Table 7.

Seventeen of the whole blood results and 9 of the sputum results were removed from the study, because either a culture was not performed (3 whole blood and 6 sputum specimens), the extraction control failed (10 whole blood and 2 sputum specimens), or the sample was not tested (4 whole blood and 1 sputum specimens). As a result of this study, the extraction control procedure was modified.

Using the standard testing protocol, all 132 whole blood and 36 sputum samples gave the expected negative result with the Plague Target 1 assay; however, 2 samples gave false positive results with the Plague Target 2 assay. All culture results were negative for *Y. pestis*. Therefore, the clinical specificity of the JBAIDS Plague Detection Kit when testing whole blood specimens is at least 97% (97–100% with 95% confidence) for the Target 1 assay and 95% (95-100% with 95% confidence) for the Target 2 assay. The clinical specificity when testing sputum specimens is at least 92% (92–100% with 95% confidence).

Table 7. Summary of Clinical Testing for Whole Blood and Sputum.

JBAIDS Plague Detection Results										
		Positive		Negative		Inhibited		Uncertain		Removed from Study	Total Subjects
		T1	T2	T1	T2	T1	T2	T1	T2		
Blood Culture—no organism		0	2	116	114	0	0	0	0	13[a]	129
Blood Culture—with organism[b]		0	0	16	16	0	0	0	0	4	20
All Patients	Site 1[c]	0	0	30[d]	30[d]	0	0	0	0	12	42
	Site 2[e]	0	2[f]	86[g]	84[h]	0	0	0	0	4	90
	Site 3[c]	0	0	16[i]	16[g]	0	0	0	0	1	17
Total		0	2	132	130	0	0	0	0	17	149
Sputum Culture—no organism		0	0	23	23	0	0	0	0	8[i]	31

Sputum Culture—with organism[j]		0	0	13	13	0	0	0	0	1	14
All Patients	Site 1	0	0	4	4	0	0	0	0	4	8
	Site 2	0	0	26[d]	26[i]	0	0	0	0	4	30
	Site 3	0	0	6	6	0	0	0	0	1	7
Total		0	0	36	36	0	0	0	0	9	45

[a] For three subjects no blood culture was performed.
[b] List of organisms recovered from blood culture and number: beta-hemolytic *Streptococcus* (n=1), *Brucella* sp. (n=2), *Corynebacterium* sp. (n=1), coagulase negative *Staphylococcus* (n=1), *Enterococcus* sp. (n=2), *Escherichia coli* (n=1), gram positive coccus (n=1), *Kocuria varians* (n=3), *Micrococcus* sp. (n=1), *Salmonella enterica* serovar Typhi (n=6), *Propionibacterium acnes* (n=1), *Staphylococcus epidermidis* (n=2), *Staphylococcus warner* (n=1), *Streptococcus constellatus* (n=1)
[c] Blood culture technique at Sites 1 and 3: BacT/ALERT® 3D using BacT/ALERT SA (aerobic) and BacT/ALERT SN (anaerobic), BACTEC™ 9240 Plus held for 5 days at 35°C.
[d] Two samples required retesting to achieve the final result.
[e] Blood culture technique used at Site 2. BACTEC Plus Aerobic F blood culture bottles held at 37°C for 5 days, manual evaluation and subculture.
[f] Two sample gave false positive results for the Target 2 assay due to non-specific amplification.
[g] Three samples required retesting to achieve the final result.
[h] Fifteen samples required retesting to achieve the final results.
[i] Seven samples required retesting to achieve the final result.
[j] For six subjects, no sputum culture results were available.
[k] List of organisms recovered from sputum culture and number: *Aspergillus fumigatus* (n=1), *Candida* sp. (n=3), *Klebsiella pneumoniae* (n=1), *Haemophilus influenza* (n=1), *Haemolytic streptococcus* (n=3), methicillin-resistant *Staphylococcus aureus* (n=5), *Moraxella cataralis* (n=1), *Pseudomonas aeruginosa* (n=1), *Serratia marcescens* (n=3), *Staphylococcus epidermidis* (n=1)
[l] Eight samples required retesting to achieve the final result.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

Not applicable, qualitative test.

N. Instrument Name:

JBAIDS instrument

O. System Descriptions:

1. Modes of Operation:
   All sample testing is managed by the software embedded Diagnostic Wizard; up to 32 capillaries can be loaded into one carousel for a run.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes _____X_____ or No _____

3. Specimen Identification:
   Specimen information is a pre-defined protocol that guides a user through entering sample information, loading the JBAIDS carousel, and starting the testing sequence. Data, result call displays and generation of final reports is managed through the traceable database using the Diagnostic Wizard. Capillaries are identified by their position number in the carousel.

4. Specimen Sampling and Handling:
   Blood specimens must be collected in tubes with sodium citrate anticoagulant. Other anticoagulants are not indicated and may contribute to unreliable results. DNA is initially extracted and purified from whole blood specimens using the Idaho Technology **IT *1-2-3*™ FLOW Sample Purification Kit** (or validated equivalent), and from blood culture and direct culture samples using the Idaho Technology **IT *1-2-3*™ SWIPE Sample Purification Kit** (or validated equivalent). Purified samples must be diluted prior to adding to reagent vials.

5. Calibration:
   The fluorimeter is factory-calibrated. Internal self-check procedures are run with each startup. Cycling temperatures are monitored continuously during a run. The internal control and positive control must meet specific criteria for a successful run.

6. Quality Control:

   Negative Control: The Negative Control (NC) is used to detect contamination from target-specific amplicon, genomic DNA {as found in the Positive Control (PC) vials}, or organism. One NC (resulting in two capillaries) must be included for each assay in a test run. Both of the NC capillaries must be Negative, or the JBAIDS software will assign Invalid results to all of the associated samples, and the run must be repeated. Frequent or repeated failures of NCs may indicate significant contamination of the work area.

   Positive Control: The PC serves as an amplification and detection control. One PC (resulting in two capillaries) must be included for each assay in a test run. Both of the PC capillaries must be Positive and have Cp results that are earlier

than the assay's specific cutoff value. If either capillary fails, the JBAIDS software will assign Invalid results to all of the associated samples, and the run must be repeated. Failure of the PCs may indicate errors in sample setup, degradation of the reagents, or a malfunction of the JBAIDS instrument. If the Inhibition Control (IC) capillaries in the same test run are Positive, then the failure is most likely caused by an isolated error with the setup of the PC. If the IC capillaries are also negative, possible causes for failure are 1) a systematic error in sample setup, 2) degradation of the reagents, or 3) a malfunction of the JBAIDS instrument.

Inhibition Control: The IC is used to demonstrate that the purified sample does not interfere with, or inhibit, the PCR reaction. Each purified sample is tested using an Unknown vial and IC vial (for a total of four capillaries). If either of the IC amplification curves demonstrates inhibition and the target assay is negative, then the result for that sample will be Inhibited. The IC amplification curves are called Inhibited by the JBAIDS software if 1) the Cp value for the IC exceeds the assay specific cutoff value or 2) if the shape of the amplification curve demonstrates reduced PCR efficiency, as evidenced by a flattening of the amplification curve.

DNA Extraction Control: Well characterized strains of *Y. pestis* and non *Y. pestis* may be used as external NCs and PCs according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. However, due to safety and regulatory requirements related to the distribution of *Y. pestis* and other select agents, this is not feasible in most user settings. To address the lack of appropriate control materials, Idaho Technology has developed a DNA Extraction Control Kit that can be used to ensure the proper functioning of the sample purification kits and procedures. The DNA Extraction Control kit is designed to work with all of Idaho Technology's 1-2-3 Sample Purification Kits and can be used for both environmental and diagnostic applications. This kit can also be used for training or for verification of user technique.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Carryover and Cross Contamination: Potential carryover was assessed in two ways for whole blood and sputum: a) Carryover during sample purification independent of reaction set-up - Positive and negative samples were purified in an alternating pattern, and the PCR reactions for positive and negative samples were then set up separately; b) Carryover during reaction set up independent of sample purification – Purified positive samples were used to set up reactions alternately with water (true negative).

Sample sets consisting of seven negative samples and seven positive samples were processed using the appropriate IT 1-2-3 sample purification kit and tested using both assays of the JBAIDS Plague Detection Kit. Positive samples were spiked with inactivated *Y. pestis* at approximately 5×10^6 CFU/mL. For each matrix evaluated, three different operators tested independent samples sets for a total of 21 positive and

21 negative samples. For blood culture specimens, only one evaluation was done. Positive and negative samples were alternately purified before being used to alternately set up positive and negative reaction.

For whole blood specimens, all 21 negative samples gave negative results with both the Target 1 and Target 2 assays. For the 21 negative sputum specimens, one sample gave a false positive result with the Target 1 assay but was negative for the Target 2 assay. Another sample gave uncertain results for both assays, and a third sample gave a negative result with the Target 1 assay and an uncertain result with the Target 2 assay. For blood culture samples, one sample gave an uncertain result with the Target 1 assay while all 21 were negative with the Target 2 assay.
Comparison of all data showed that the *Y. pestis* Target 1 assay is more likely to detect sample purification carryover than the *Y. pestis* Target 2 assay.

Sample Transport and Storage

The evaluations were performed using simulated samples prepared by spiking live *Y. pestis* into whole blood and sputum specimens. For the assessment of colonies and blood-culture specimens, a panel of *Y. pestis* and non- *Y. pestis* isolates was used for the positive and negative samples, respectively.

Q.   Proposed Labeling:

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

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