Draft Guidance for Industry and Food and Drug Administration Staff

---

Class II Special Controls Guidance Document: *In Vitro* Diagnostic Devices for *Yersinia* spp. Detection

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Document issued on: November 7, 2011

DRAFT

You should submit comments and suggestions regarding this draft document within **90** days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Submit electronic comments to http://www.regulations.gov. Identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this document contact Beena Puri, Ph.D., Division of Microbiology Devices, at 301-796-6202 or by email: beena.puri@fda.hhs.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health
Office of *In Vitro* Diagnostic Device Evaluation and Safety
Division of Microbiology Devices

Preface

Additional Copies

Additional copies are available from the Internet. You may also send an e-mail request to dsmica@fda.hhs.gov to receive an electronic copy of the guidance or send a fax request to 301-827-8149 to receive a hard copy. Please use the document number (1714) to identify the guidance you are requesting.

DRAFT

Table of Contents

1. INTRODUCTION 4

2. *YERSINIA* SPP. - BACKGROUND 4

3. PREMARKET NOTIFICATIONS - BACKGROUND 5

4. SCOPE 6

5. ISSUES OF SAFETY AND EFFECTIVENESS REQUIRING SPECIAL CONTROLS 6

6. DEVICE DESCRIPTION 7

7. PERFORMANCE STUDIES 9

8. LABELING 12

9. LIMITED DISTRIBUTION 14

10. SPECIFIC CONSIDERATIONS FOR *YERSINIA* SPP. DEVICES USING NUCLEIC ACID AMPLIFICATION 15

11. REFERENCES 21

DRAFT

Draft Guidance for Industry and Food and Drug Administration Staff

Class II Special Controls Guidance Document: *In Vitro* Diagnostic Devices for *Yersinia* spp. Detection

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

DRAFT

1. Introduction

This draft special controls guidance document was developed to support the proposed rule classifying *in vitro* diagnostic device for *Yersinia* spp. detection, a previously unclassified preamendments device, into class II, as recommended by the Microbiology Devices Advisory Panel on March 7, 2002 and designating limitations on distribution and this guidance document as the special controls.

When the rule is finalized, designation of this guidance document as a special control means that any firm currently marketing, or intending to market, *in vitro* diagnostic devices for *Yersinia* spp. detection will need to address the issues covered in the special controls guidance. The firm will need to show that its device addresses the issues of safety and effectiveness identified in the guidance, either by meeting the recommendations of the guidance or by some other means that provides equivalent assurances of safety and effectiveness.

After a final rule becomes effective, the device must comply with the limitation on distribution specified as one special control in the classification regulation. (See proposed 21 CFR 866.3945(b)(2) and sections 3 and 8 of this document.)

2. *Yersinia* spp. - Background

An *in vitro* diagnostic device for *Yersinia* spp. detection is used to detect and differentiate among *Yersinia* spp. and presumptively identify *Yersinia pestis* and other *Yersinia* spp. from cultured

isolates or clinical specimens, as an aid in the diagnosis of plague and other diseases caused by *Yersinia* spp. This device may consist of *Yersinia* spp. antisera conjugated with a fluorescent dye (immunofluorescent reagents) used to presumptively identify Yersinia-like organisms in clinical specimens; or bacteriophage used to differentiate *Yersinia pestis* from other *Yersinia* spp. based on susceptibility to lysis by the phage; or antigens used to identify antibodies to *Yersinia pestis* in serum. This draft guidance includes recommendations for satisfying the proposed requirement of special controls for all devices of this type, including both the preamendments technologies described above and nucleic acid amplification-based *Yersinia pestis* assays, one of which has been determined to be substantially equivalent to other devices within this type through the premarket notification (510(k)) process and thus would also be classified as class II devices and subject to special controls under the proposed classification.

Plague caused by *Y. pestis* is a disease of humans and animals. There are three different forms of plague in humans: bubonic, pneumonic, and septicemic depending on the portal of bacteria entry. The most common form is bubonic plague, which results from entry of the organism through a bite from an infected flea or exposure to infected material through a break in the skin. Inhalation of aerosolized *Y. pestis* results in pneumonic plague, which is the most lethal and aggressive form of the disease and can spread from person to person. Septicemic plague occurs when plague bacteria multiply in the bloodstream. This form of plague can be contracted when bacteria transmitted by a fleabite enter directly into the bloodstream or as a complication of bubonic or pneumonic plague. Neither bubonic plague nor septicemic plague can spread directly from person to person. *Y. pseudotuberculosis* and *Y. enterocolitica* are causative agents of gastrointestinal disease that can occur by ingestion of contaminated meat or other food products. Only *Y. pestis* causes acute and fatal disease if the infection is untreated.

DRAFT

3. Premarket Notifications - Background

FDA believes that special controls, when combined with the general controls, will be sufficient to provide reasonable assurance of the safety and effectiveness of *in vitro* diagnostic devices for *Yersinia* spp. detection. Designation of this guidance document as a special control means that a manufacturer who intends to market a device of this type should (1) conform to the general controls of the Federal Food, Drug, and Cosmetic Act (the FD&C Act), including the premarket notification requirements described in 21 CFR 807 Subpart E, (2) address the specific issues of safety and effectiveness identified in this guidance document, (3) satisfy the other special control designated in 21 CFR 866.3945(b), and (4) obtain a substantial equivalence determination from FDA prior to marketing the device.

This draft guidance document identifies the proposed classification regulation and associated product codes for *in vitro* diagnostic devices for *Yersinia* spp. detection (refer to **Section 4 - Scope**). In addition, other sections of this draft guidance document list the issues of safety and effectiveness and describe measures that, if followed by manufacturers and combined with the general controls, will generally address the issues associated with these devices and lead to a timely premarket notification [510(k)] review and clearance. This draft document, when final, will supplement other FDA documents regarding the specific content requirements of a premarket notification submission. You should also refer to 21 CFR 807.87 and CDRH's Device Advice http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm.

4. Scope

The scope of this document is limited to devices as described in proposed 21 CFR 866.3945, which have the following product codes:

OIH [Assay, Nucleic Acid Amplification, *Y. pestis*]

This draft guidance is *not* intended to address specific issues for testing environmental samples.

§ 866.3945 *In vitro* diagnostic device for *Yersinia* spp. detection.

(a) Identification. An *in vitro* diagnostic device for *Yersinia* spp. detection is a device that is used to detect and differentiate among *Yersinia* spp. and presumptively identify *Yersinia pestis* and other *Yersinia* spp. from cultured isolates or clinical specimens as an aid in the diagnosis of plague and other diseases caused by *Yersinia* spp. Diseases caused by *Yersinia* infections include three different forms of plague (bubonic, pneumonic, and septicemic), caused by *Y. pestis*, and gastrointestinal infection, caused by *Y. pseudotuberculosis* and *Y. enterocolitica.* This device may consist of *Yersinia* spp. antisera conjugated with a fluorescent dye (immunofluorescent reagents) used to presumptively identify *Yersinia*-like organisms in clinical specimens; or bacteriophage used for differentiating *Y. pestis* from other *Yersinia* spp. based on susceptibility to lysis by the phage; or antigens used to identify antibodies to *Y. pestis* (Fraction 1) in serum.

In addition to this guidance document, FDA proposes that the special controls for this device include limiting distribution to laboratories with experienced personnel who have training in principles and use of microbiological culture identification methods and infectious disease diagnostics, and with appropriate biosafety equipment and containment. (See proposed 21 CFR § 866.3945(b) and **Section 9**, **Limited Distribution.**)

5. Issues of Safety and Effectiveness requiring special controls

FDA has identified the risks of a false negative test result and the risks of a false positive test result, both of which can lead to individual and public health consequences, as issues of safety and effectiveness associated with this device that require special controls. In addition, FDA has identified the health risks to laboratory workers, which may be associated with handling specimens and control materials, as requiring special controls. These issues and the location of recommendations for addressing them are summarized in the table below.

To elaborate, failure of *Yersinia* spp. devices to perform as indicated or an error in interpretation of the results may lead to misdiagnosis and improper patient management or to inaccurate epidemiological information that may contribute to inappropriate public health responses. A false positive result may lead to a medical decision causing a patient to undergo unnecessary or ineffective treatment, as well as inaccurate epidemiological information on the presence of

DRAFT

plague disease in a community. A false negative result may lead to delayed recognition by the physician of the presence or progression of disease and inaccurate epidemiological information to control and prevent additional infections. A false negative result could also potentially delay diagnosis and treatment of infection caused by *Yersinia pestis* or other *Yersinia* spp.

Exposure to organisms potentially present in test specimens and those used as control materials poses a risk of infection to laboratory workers. Consequently, FDA proposes that use of *Yersina spp.* detection devices be restricted to laboratories with experienced personnel who have training in principles and use of microbiological culture identification methods and infectious disease diagnostics and with appropriate biosafety equipment and containment.

In the table below, FDA has identified the issues requiring special controls. The measures recommended to mitigate these identified issues are in this guidance document, as shown in the table below, in combination with proposed subsection 21 CFR 866.3945(b)(2). We recommend that you also conduct a risk analysis, prior to submitting your premarket notification, to identify any other risks specific to your device. The premarket notification should describe the risk analysis method. If you elect to use an alternative approach to address a particular risk identified in this guidance document or have identified risks additional to those in this document, you should provide sufficient detail to support the approach you have used to address that risk.

Identified Risks	Mitigation Measures
A false negative test result may lead to delay of therapy and progression of disease and epidemiological failure to promptly recognize disease in the community	Section 6 (Device Description) - Recommended Section 7 (Performance Studies) - Recommended Section 8 (Labeling) - Recommended Section 9 (Limited Distribution) - Required
A false positive test result may lead to unnecessary treatment and incorrect epidemiological information that leads to unnecessary prophylaxis and management of others	Section 6 (Device Description) - Recommended Section 7 (Performance Studies) - Recommended Section 8 (Labeling) - Recommended Section 9 (Limited Distribution) - Required
Biosafety and a risk of transmission of Yersinia infection to laboratory workers handling test specimens and control materials	Section 8 (Labeling) - Recommended Section 9 (Limited Distribution) - Required

6. Device Description

Key elements of a 510(k) submission are the intended use, the type of specimens tested, the technological characteristics of your device, and a legally marketed predicate device that you will compare with your device. Additionally, you should identify the regulation and the product

code. In order to help FDA review your submission efficiently, we recommend that you include a table including similarities and differences between the predicate and your device. We encourage you to reference appropriate peer-reviewed articles that support the use of your device for its intended diagnostic use and the specific test principles incorporated into the device design. We recommend that you describe each of these device elements in detail.

Furthermore, we recommend that you include the following descriptive information to adequately characterize your device for the detection of *Yersinia* spp.

Intended Use

Your 510(k) must include proposed labeling that describes the intended use of your product (see 21 CFR 807.87(e)). Your 510(k) must specify the measurand the assay measures (for example, *Yersinia pestis* cell surface protein or target DNA sequences from specific *Yersinia pestis* plasmids) (see 21 CFR 807.87(e) and 21 CFR 807.92(a)). You should clearly state the clinical indications for which the test is to be used and the specific population for which the test is intended. You should include a clinical and demographic description of patients (e.g., sex, age, symptoms) for whom clinical performance has been demonstrated. The intended use should specify whether the test is qualitative or quantitative (see 21 CFR 807.87(e) and 21 CFR 807.92(a)). You should ensure that all elements of the intended use are clearly stated, including specific conditions of use such as type of specimens to be tested, for example, whole blood collected in sodium citrate from individuals suspected of having plague, positive blood cultures, or cultured organisms grown on blood agar.

DRAFT

You should also prominently provide the following statement immediately below your intended use: "For use in laboratories with experienced personnel who have training in principles and use of microbiological culture identification methods and infectious disease diagnostics, and with appropriate biosafety equipment and containment."

Reagents and other device components

You must describe reagents and other device components in your labeling (see 21 CFR 809.10). We recommend that you follow general guidance provided in other FDA guidance documents.

Testing Procedures using your device

In your 510(k), you should describe, in detail, the principles of operation applicable to your device for its intended use. We recommend that you specifically describe testing conditions, procedures and controls designed to provide safeguards for conditions that can cause false positive and false negative results or present a biosafety hazard. These descriptions include, but are not limited to, procedures, methods, and practices incorporated into your directions for use (see **Section 8 - Labelin**g) to mitigate risks associated with testing [Ref. 1].

Specimen Storage and Shipping Conditions

If you recommend specimen storage conditions, you should demonstrate that your device generates equivalent results for the stored specimens at several time points throughout the duration of the recommended storage and at both ends of your recommended temperature range. If a transport medium is recommended for storage or shipping, you should conduct appropriate studies to demonstrate that the device will perform as described when the specimen is preserved in the transport medium.

Interpreting Test Results/Reporting

In your 510(k), we recommend that you describe how presumptive positive, equivocal, and negative results are determined and how they should be interpreted, if applicable. You should provide clear explanations for how interpretative algorithms have been determined.

7. Performance Studies

We recommend that you evaluate the following performance characteristics, in order to document performance and properly label your device in conformance with 21 CFR 809.10(b)(12). You should describe the studies in your 510(k) and clearly summarize results, preferably in tabular form where applicable. Relevant findings in published literature may be cited. Specific additional guidance for devices used in molecular diagnostic test methods and for devices used in immunological test methods can be found in CLSI/LA 18-A2 **Section 11** [Ref. 2].

DRAFT

In order to review data from your studies, we recommend that you provide specific details of the study protocols used to generate data. These specifics are also helpful to aid users in interpreting performance data in your labeling. When referring to Clinical and Laboratory Standards Institute (CSLI) protocols or guidelines, we recommend that you indicate which specific aspects of the protocols or guidelines you followed.

A. Analytical/Laboratory Performance Studies

The appropriate types of analytical studies will depend on the applied technology, principles of operation and scientific evidence available. The following are some pertinent examples. These are not intended as an all-inclusive list. Additional types of analytical data may be appropriate, depending on the device type.

(1) Determination of assay/reagent specificity

For devices used to identify *Yersinia pestis* from cultures, we recommend that you characterize assay performance for bacteriophage and immunofluorescent antibody reagents using at least 25 different strains of *Y. pestis* (representing geographic and temporal diversity, including known genotypic and phenotypic variants), 10 strains of *Y. pseudotuberculosis*, and 10 strains of *Y. enterocolitica,* along with other representative *Yersinia* spp. and non-*Yersinia* Gram negative rods. If your device is indicated for direct specimen testing methods, you should also include organisms that could be expected to

be found at the sampling site (e.g. both normal throat flora and other potential pathogens). Strains used for characterization of assay/reagent specificity can be selected from well-characterized archives or repositories. Definitive species identification of *Yersinia* spp. may call for a combination of phenotypic and genotypic methods (e.g., biochemical, antigenic, morphology, plasmid characterization, genotyping).

We recommend that you evaluate antigens for anti-*Yersinia pestis* antibody testing using human sera from naturally infected humans and those immunized with plague vaccine. Sera from at least 200 human samples, including those from individuals with compatible diseases and conditions, should also be tested for specificity.

(2) Precision/Reproducibility

We recommend that you characterize within-day, day-to-day, intra-laboratory, and inter-laboratory reproducibility. The reproducibility panel should consist of samples around the cutoff of the assay, at low and moderate positive concentrations, and at high negative concentrations. If results of your device are interpreted visually, you should also conduct between-operator reproducibility. If your device uses an instrument, then you should also conduct precision studies with all instruments recommended for use with your device [Ref. 3].

(3) Interfering/inhibitory substances

DRAFT

We recommend that you provide information and data to demonstrate that potentially interfering or inhibitory substances encountered in specific specimen types do not affect results. See Table 1 for a list of potentially endogenous and exogenous interfering substances that could be present in clinical specimens, e.g., blood, sputum, culture, etc. If interference or inhibition has been reported in the literature or is evident in your studies, you should provide validated procedures or methods that can be used to avoid erroneous results.

Table 1. List of Evaluated Potentially Interfering Substances

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

	Naproxen sodium Rifampin Streptomycin Sulfamethoxazole Tetracycline Tobramycin Trimethoprim		N-acetylcysteine Blood culture media Sheep blood agar media
Solvents*	Technique-specific Substances		
Acetone DMSO Ethanol NH4OH	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.

Your studies should include the effect of culture age and growth media with specific antibody and nucleic acid reagent testing from cultures (solid or liquid). You should include the results in the package insert in the Performance Characteristics section and also as a Limitation statement to inform the user that cultures older than a specified number of days may result in a false negative result.

DRAFT

(4) Effect of culture inoculation density on results with bacteriophage reagents (bacteriophage assays using culture plating methods)

We recommend that you conduct studies to demonstrate whether there is a risk of a false negative test result due to heavy culture inoculation when using bacteriophage-specific assays. One technique may be to streak a suspect culture for isolation and add bacteriophage to areas of the plate with varying amounts of inoculum. You should also assess phage titer and stability of bacteriophage reagents.

B. Clinical Information

This guidance addresses clinical specimens collected from individuals with suspected infection.

You should provide information to demonstrate the reliability of your device for detecting *Yersinia* spp. in each type of clinical specimen that you indicate as suitable for testing with your device. In general, when the number of human clinical samples available for clinical testing is very low or non-existent, the available evidence for FDA's premarket review may, of necessity, be obtained from analytical studies as well as retrospective clinical studies; spiked human samples for sensitivity studies may be adequate. In this circumstance, it is particularly critical to have well designed analytical studies. Animal studies are optional and can be used to supplement analytical studies where appropriate. Performance assessments should be relative to the known

presence or absence of a characterized *Yersinia* spp. or to the definitive identification of culture growth. Multiple tests and methods may be needed to appropriately identify/characterize a *Yersinia* spp. recovered from human specimens by culture methods or detected directly in human specimens. We recommend that you contact the Division of Microbiology devices before beginning your studies in order to discuss appropriate study design options.

We recommend that you also provide data from testing specimens from the intended use population (e.g., patients with febrile illnesses or skin lesions). Because plague would not be expected in a prospective evaluation, these data should not be represented as specificity, but rather as agreement with an expected negative result.

For devices used to identify culture isolates or growth, laboratory evaluations are sufficient, as long as studies with culture stocks reasonably represent fresh culture growth and conditions for testing (e.g., 12-18 h growth from 5% sheep blood agar plates).

8. Labeling

IVD devices for *Yersinia* spp. detection, like other devices, are subject to statutory requirements for labeling (The FD&C Act, Sections 502(a), 201(n); 21 USC §§ 352(a), 321(n)). Labeling for these devices must provide adequate directions for use and adequate warnings and precautions. (The FD&C Act, Section 502(f); 21 USC § 352(f)). Specific labeling requirements for all IVD devices are set forth in 21 CFR 809.10.

DRAFT

The 510(k) submission must include proposed labeling in sufficient detail to satisfy the requirements of 21 CFR 807.87(e). Final labeling for an *in vitro* diagnostic device must comply with the requirements of 21 CFR 809.10 before being introduced into interstate commerce; however, submission of final labeling is not required for 510(k) clearance.

To ensure compliance with section 502 of the FD&C Act and 21 CFR. 809.10, FDA recommends that labeling for IVD devices for *Yersina* spp. detection address the items identified below. These labeling recommendations also help to mitigate the risks identified previously in this guidance and thus help to ensure safe and effective use of these devices.

A. Intended Use

A clear intended use statement is critical. We recommend that you incorporate into the intended use statement the intended specimen type(s), whether the testing to be performed is qualitative, semi-quantitative, or quantitative, and the testing methodology along with the indicated patient population and other conditions for use.

- You should also prominently provide the following statement immediately below your intended use:

  > "For use in laboratories with experienced personnel who have training in principles and use of microbiological culture identification methods or infectious disease diagnostics and with appropriate biosafety equipment and containment."

B. Directions for Use

We recommend that you address the following in the directions for use:

- Emphasize appropriate storage conditions for reagents, and identify reagents that are temperature, humidity, and/or light sensitive.

- When testing requires the culture of *Yersinia* spp., specify the appropriate type of culture media from which growth is to be tested, incubation conditions, and length of incubation (including minimum and maximum incubation times).

- Provide directions for using control reagents provided with the product, as well as required or recommended control materials that may be used but are not provided. Describe each aspect of the testing procedure that is controlled. Provide acceptable values for the control reagent testing and justification for your selected values.

- Provide guidance for biosafety precautions with specimen handling and testing procedures. Specify at which procedural step the test sample is non-infectious.

- For products that rely on antigen/antibody reactions in the testing procedure, include recommendations for reducing the risk of a false negative test result due to prozone or Hook effect.

DRAFT

C. Precautions

We recommend that you include the following type of statement in the Precaution Section of the package insert: "The interpretation of test results requires experienced clinical personnel who have training in principles and use of microbiological culture identification methods or infectious disease diagnostics and have the necessary awareness to report an identification of *Y. pestis* and coordinate with local or state public health directors."

D. Interpretation and Reporting of Assay Results

We recommend that you include the following:

- We recommend that you define each of the possible testing results: positive, equivocal or indeterminate, and negative. You should also describe how the operator should interpret these test results, and give acceptance/rejection criteria for controls. Include suggestions for how to proceed if control results are not acceptable.

- We recommend that you provide clear and exact criteria for evaluating a test result as positive, negative, or equivocal/indeterminate. We recommend using photographs and/or diagrams to indicate how to interpret results for tests that give a qualitative result. Additionally, we recommend that you provide an adequate description of the expected

results based on the likelihood of a *Y. pestis* identification (for culture identification reagents), the likelihood of infection (for reagents detecting specific anti- *Y. pestis* antibodies), or the likelihood of *Y. pestis* presence (for reagents detecting *Y. pestis* directly in patient specimens). This information can be supported by reliable information available from the literature or other sources.

- For tests that rely on antigen/antibody interactions to detect bacterial cell components or bacterial products, you should include recommendations for reducing the risk of a false negative test result due to non-optimal initial inoculum density.

- For tests to detect an antibody response to *Yersinia pestis*, you should include a warning statement concerning the interpretation of a positive test and how it does not by itself conclusively establish recent infection, as persons immunized with a plague vaccine may test positive with this test in the absence of any natural exposure to *Yersinia pestis*. Labeling should also indicate that antibiotic treatment early in natural infection with *Yersinia pestis* may decrease the antibody response and therefore may give a negative result with this test.

Your labeling should include a statement that *Yersinia pestis* is a Nationally Notifiable Disease[1] that must be reported to public health authorities in accordance with state and local law. Users should verify reporting requirements in their area and notify their state or local public health laboratory, the Centers for Disease Control and Prevention, and any other Agency specified by their accreditation guidelines if *Yersinia pestis* or plague is suspected.

DRAFT

E. Performance Characteristics

We recommend that you include in the package insert a summary of the study designs and the results of the studies described in Section 6 that would aid users in interpreting test results. This includes clinical and analytical performance characteristics. If the assay was not evaluated using specimens from individuals presenting with signs and symptoms of plague, users should be instructed to establish the clinical sensitivity of this test on prospectively collected clinical specimens as these specimens become available. Data for negative agreement/clinical specificity should be included and indicated clearly to the users.

9. Limited distribution

As will be required by the special control if proposed 21 CFR 866.3945(b)(2) is finalized, distribution of *in vitro* diagnostic devices for *Yersinia* spp. detection is limited to laboratories

[1] See (1) Center for Disease Control and Prevention Morbidity and Mortality Weekly Report, March 21, 2008, The *Summary of Notifiable Diseases — United States,*
(2) Maryland Department of Health & Mental Hygiene Plague Fact Sheet for Health Care Providers (http://www.edcp.org/guidelines/plague_hcp.cfm)
(3) Center for Disease Control Summary of Notifiable Diseases (http://www.cdc.gov/osels/ph_surveillance/nndss/annsum/1998/98hilites.htm)

with experienced personnel who have training in principles and use of microbiological culture identification methods or infectious disease diagnostics and with appropriate biosafety equipment and containment.

10. Specific considerations for *Yersinia* spp. Devices using Nucleic Acid Amplification

Yersinia spp. detection devices that employ Nucleic Acid Amplification are used to determine the presence of pathogenic *Yersinia* spp. directly in human specimens and/or blood or colony cultures derived from clinical specimens by detecting nucleic acid sequences or regions that are unique to *Yersinia* spp. and that discriminate *Yersinia* spp. pathogen from other microbial organisms. These devices include primers, probes, enzymes, and specific controls for amplification and are designed for use in specific instrument systems. Detection of *Y. pestis* by a nucleic acid amplification detection system aids in the definitive identification of *Y. pestis* in infected patients in conjunction with other laboratory results and clinical presentation. The following are specific considerations applicable to this type of IVDs for *Yersinis* spp. detection.

A. Reagents and other device components

Nucleic Acid Amplification-based devices are intended for presumptive detection of *Y. pestis* DNA in human specimens or blood or colony culture or liquid culture derived from clinical specimen. We recommend that you describe design requirements for your device that address or mitigate risks associated with primers, probes, instruments, and controls used in a nucleic-acid test procedure to detect targeted DNA segments from *Y. pestis.* Some examples are given below:

DRAFT

- Designing your freeze-dried sets of reagents or any other closed tube test system (e.g. self containing cartridge) to minimize false positives due to contamination or carryovers.
- Designing one or more than one assay for targeting different DNA sequences unique to *Y. pestis*
- Developing positive controls, negative controls, and inhibition controls to ensure accurate test results
- Developing methods for extraction and purification that yield suitable quality and quantity of DNA from human specimens or in blood or colony cultures or liquid culture derived from clinical specimens for use in the test system with your reagents.
- Optimizing your reagents and test procedures for recommended instruments.
- Including illustrations or photographs of any non-standard equipment or methods if applicable.

In your 510(k), you should provide performance information supporting the conclusion that your design requirements have been met. We recommend that you provide the rationale for selection of specific DNA target sequences and selection of primers and probes (See **Section 7 – Performance Studies**).

The specific extraction method recommended for each specimen type should be listed by name and catalog number in the package insert of your device (See **Section 8 – Labeling**).

B. Testing Procedures

We recommend that you include the following information in your 510(k) application:

- Overall design of the testing procedure, including control elements incorporated into the recommended testing procedures. These controls should approximate the lower range of clinically relevant *Yersinia* DNA levels and should be processed in the same manner as a clinical sample.
- Descriptions of or recommendations for additional external or internal positive and negative controls that monitor for contamination and extraction efficiency (e.g. an internal control, as a control for nucleic acid extraction and inhibition).
- Features and additional controls that reduce failure to recognize procedural errors or factors (e.g., degradation of master mix) that adversely affect amplification and detection conditions.

C. Controls

When conducting the performance studies described below, we recommend that you run appropriate external controls every day of testing for the duration of the analytical and clinical studies. You may contact OIVD's Division of Microbiology Devices at FDA for further information regarding controls. For devices based on nucleic acid technology, we generally recommend that you include the following types of controls:

DRAFT

(1) Negative Controls

Blank or no template control

The blank, or no-template control, contains buffer or sample transport media and all of the assay components except nucleic acid. This control is used to rule out contamination with target nucleic acid or increased background in the amplification reaction. It may not be needed for assays performed in single test disposable cartridges or tubes.

Negative sample control

The negative sample control contains non-target nucleic acid or, if used to evaluate extraction procedures, it contains the whole organism (other than *Y. pestis*). It reveals non-specific priming or detection and indicates that signals are not obtained in the absence of target sequences. Examples of acceptable negative sample control materials include:

- Patient specimen from a non- *Y. pestis* infected individual
- Samples containing a non-target organism

(2) Positive Controls

Positive control for complete assay

The positive control contains target nucleic acids and is used to control the entire assay process including DNA extraction, amplification, and detection. It is designed to mimic a patient specimen and is run as a separate assay, concurrently with patient specimens, at a frequency determined by a laboratory's Quality System (QS). Examples of acceptable positive assay control materials include:

- Patient specimen from a *Y. pestis* infected individual or spiked matrices with live *Y. pestis*
- *Y. pestis* culture isolates

Positive control for amplification/detection

The positive control for amplification/detection contains purified target nucleic acid at or near the limit of detection for a qualitative assay. It controls the integrity of the sample and the reaction components when negative results are obtained. It indicates that the target is detected if it is present in the sample.

(3) Internal Control

The internal control is a non-target nucleic acid sequence that is co-extracted and co-amplified with the target nucleic acid. It controls for integrity of the reagents (polymerase, primers, etc.), equipment function (thermal cycler), and the presence of amplification inhibitors in the samples. Examples of acceptable internal control materials include human nucleic acid co-extracted with the *Y. pestis* DNA and primers amplifying human housekeeping genes (e.g. RNaseP, β-actin). The internal control for a device is determined on a case-by-case basis [Ref. 4].

DRAFT

D. Performance Studies

For studies intended to determine the performance characteristics of nucleic acid amplification-based devices, we recommend that you include in the 510(k) application the information described below. This section complements the recommendations for performance studies described earlier in this document.

(1) Nucleic acid extraction

Different extraction methods may yield *Y. pestis* DNA of varying quantity and quality, and therefore the extraction method can be crucial to a successful result. Purification of *Y. pestis* DNA from blood, sputum, or any other specimen or liquid blood culture or colony culture specimens can be challenging because biological samples may contain low bacterial loads in the background of human genomic DNA, as well as high levels of proteins and other contaminants.

For these reasons, you should evaluate the effect of your chosen extraction methods on the performance of the assay with respect to satisfactory *Y. pestis* DNA quantity and quality for the intended use of the assay. In addition, you should evaluate your assay's analytical and clinical performance characteristics using the entire analytical process (including extraction procedures) that you recommend for use with your assay. This should include demonstrating the Limit of

Detection (LoD) and reproducibility of your assay with each extraction procedure. Recommendations for conducting the LoD study are provided under "Limit of Detection" (See **Section 10D(4) – Analytical Sensitivity**). In addition, external site studies (including reproducibility and clinical studies) should include the extraction procedures prescribed in your labeling.

We recommend that you perform these evaluations whether you intend to actually provide reagents in your test kit for extraction and preparation of nucleic acid or whether you simply instruct users concerning appropriate reagents.

If you recommend or include multiple extraction methods, you should demonstrate the LoD and reproducibility for each method. With the assumption that the extraction method introduces minimum variability to the overall assay performance, you may be able to combine the extraction method variable with each site performance variable. For example if you recommend three different extraction methods, you can design a reproducibility study by evaluating one of the three extraction methods at each testing site: test extraction method A at site 1, method B at site 2, and method C at site 3. If the results generated from the test panel mentioned above do not show significant differences, no further reproducibility studies are needed. However, if the initial extraction equivalency studies from the three sites indicate statistically significant differences in assay performance, the reproducibility study should be expanded to include testing each extraction method at three study sites (e.g., site 1 extraction method A, B, and C; site 2 extraction method A, B, and C; and site 3 extraction method A, B, and C).

DRAFT

In addition to the analytical studies (LoD and Reproducibility), each extraction method should be utilized in at least one clinical site during the clinical trials to generate clinical performance data. If results from the expanded reproducibility testing indicate a significant difference in efficiency among the extraction methods, the data from each clinical testing site (using a different nucleic acid extraction method) are not considered equivalent and should not be pooled, but rather should be analyzed separately. As a result, additional prospective clinical samples may be called for in order to support the claimed extraction method.

(2) Assay Cut-off

`We recommend that you should explain how the cut-off(s) was determined (see also **Section 10D(3) Interpreting Test Results/Reporting**) as well as how it was validated. The cut-off should be determined using appropriate statistical methods. To support the cutoff you determined, you may provide, for example, a result distribution, 95^{th} and 99^{th} percentiles, percents of the non-negative (positive or equivocal) results, and so on, for the clinical samples without any *Y. pestis* DNA in your pilot studies. Selection of the appropriate cut-off can be justified by the relevant levels of sensitivity and specificity based on Receiver Operating Curve (ROC) analysis of the pilot studies with clinical samples (for details about ROC analysis, see CLSI document GP10-A [Ref. 5]). If the assay has an equivocal zone, you should explain how you determined the limits of the equivocal zone. The performance of your device using the pre-determined cut-off (and equivocal zone, if applicable) should be validated in an independent population consistent with the defined intended use of your device.

(3) Interpreting Test Results/Reporting

We recommend that you describe how presumptive positive, negative, equivocal, or invalid results are determined and how they should be interpreted if applicable. There should be clear explanations for how interpretative algorithms have been determined.

We recommend that you provide the cut-off value for defining a negative result of the assay. If the assay has only two output results (negative/positive), this cut-off also defines a positive result of the assay.

If the assay has an equivocal zone, we recommend that you provide cut-off values (limits) for the equivocal zone. If your interpretation of the initial equivocal results requires retesting, you should provide (1) a recommendation whether retesting should be repeated from the same nucleic acid preparation, a new extraction, or a new patient specimen, and (2) an algorithm for defining a final result by combining the initial equivocal result and the results after re-testing (note that this algorithm should be developed before the pivotal clinical study that evaluates the clinical performance of the assay). If one of the reported outputs of your assay can be an equivocal result, you should provide the interpretation and recommendation for how the user should follow up on the equivocal results.

If the assay has an invalid result, we recommend that you describe how an invalid result is defined. If internal controls are part of the determination of invalid results, you recommend that you provide the interpretation of each possible combination of control results for defining the invalid result. You should provide recommendations for how to follow up any invalid result, i.e., whether the result should be reported as invalid or whether retesting is recommended. If retesting is recommended, you should provide information similar to that for retesting of equivocal results (i.e., whether retesting should be repeated from the same nucleic acid preparation, a new extraction, or a new patient specimen).

DRAFT

(4) Analytical Sensitivity (Limit of Detection)

We recommend that you determine the limit of detection (LoD) of your assay at the preclinical stage using approaches described in CLSI EP17-A [Ref. 6]. You should determine the lowest level of *Y. pestis* detection in appropriate specimen types using your device. The study should include testing serial dilutions of viable (live) *Y. pestis* in replicates of 3-5. Each dilution should be made using *Y. pestis* negative pooled human samples such as blood or sputum or an equivalent matrix. We recommend that you report the LoD as the level of *Y. pestis* that gives a 95% detection rate. Based on the titration results, the LoD may be further confirmed by preparing at least 50 additional replicates at the LoD concentration and demonstrating that *Y. pestis* was detected 95% of the time. The LoD should be correlated to CFU/ml and DNA copy numbers present in *Y. pestis.* The lowest level of *Y. pestis* DNA specimen should be close to the LoD concentration and correlated to CFU/mL and DNA copy numbers.

(5) Carry-Over and Cross-contamination Studies (for multi-sample assays and devices that require instrumentation.)

We recommend that you demonstrate that carry-over and cross-contamination do not occur with your device. In a carry-over and cross-contamination study, we recommend that high positive samples be used in series alternating with high negative samples in patterns dependent on the operational function of the device. We recommend that you perform at least 5 runs with alternating high positive and high negative samples. We recommend that the high positive samples in the study be high enough to exceed 95% or more of the results obtained from specimens of diseased patients in the intended use population. We recommend that the high negative samples contain the analyte concentration below the cut-off such that repeat testing of this sample is negative approximately 95% of the time. The carry-over and cross-contamination effect can then be estimated by the percent of negative results for the high negative sample in the carry-over study compared with 95%.

(6) Interference/Inhibitory Substances

We recommend that you test the effects of potentially endogenous interfering substances encountered in blood or human specimens, colony cultures, or liquid culture derived from clinical specimens and exogenous interfering substances that could be introduced during sample purification or reaction set-up. These interfering substances may interfere with assay performance. You should include the tabulated data for the evaluated endogenous and exogenous interfering substances for your device in the submission. The concentration of each substance tested should be represented at a relevant concentration in accordance with CLSI EP7-A2 [Ref. 7].

DRAFT

(7) Precision/Reproducibility/Repeatability

We recommend that you conduct within-laboratory precision studies for devices that include instruments or automated components. You should characterize within-day, day-to-day, intra-laboratory, and inter-laboratory precision. As a general guide, we recommend the following protocol for a nucleic acid amplification assay:

- that you perform reproducibility studies at three sites (two external, one in-house site).
- that you use a five day testing protocol, including, at a minimum, two runs per day (unless the assay design precludes multiple runs per day) and three replicates of each panel member per run.
- that you have at least two operators each day at each facility perform the test.

We recommend that you prepare reproducibility panels by spiking each matrix (e.g., blood, human specimens, colony cultures, or liquid culture derived from clinical specimens) with *Y. pestis* at low (near LOD), medium, or high level. We recommend that negative sample panels be unspiked specimens for each matrix.

Each panel should consist of 6-9 samples that include three levels of analyte as described below:

- A "high negative" sample with a concentration of analyte below the clinical cut-off so that retesting of this sample should be negative approximately 95% of the time.
- A "low positive" sample with a concentration of analyte just above the clinical cut-off so that repeated test results should be positive approximately 95% of the time.

- A "moderate positive" sample with a concentration that one can anticipate positive results approximately 100% of the time.

You may refer to the CLSI document EP15-A2 [Ref. 8], EP5-A2 [Ref. 3], and EP12-A2 Ref. 9] for guidance on reproducibility study design.

11. References

1. Clinical and Laboratory Standards Institute (CLSI). Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Third Edition. CLSI document H18-A3 (ISBN 1-56238-555-0). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.

2. Clinical Laboratory Standards Institute (CLSI). Specifications for Immunological Testing for Infectious Diseases; Approved Guideline – Second Edition. CLSI document I/LA18-A2 (ISBN 1-56238-445-7) CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2001.

3. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Quantitative Methods; Approved Guideline-Second Edition. CLSI document EP5-A2 (ISBN 1-56238-000-0). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.

4. Clinical and Laboratory Standards Institute (CLSI). Molecular Diagnostic Methods for Infectious Disease; Approved Guideline. CLSI document MM3-A2 [ISBN 1-56238-596-8] Clinical and Laboratory Standards Institute, Wayne Pennsylvania 19087-1898 USA, 2006.

5. Clinical and Laboratory Standards Institute (CLSI). Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristics (ROC) Plots; CLSI document GP10-A Approved Guideline: Clinical and Laboratory Standards Institute; Wayne, Pennsylvania 19087-1898 USA, 1995).

6. Clinical Laboratory Standards Institute (CLSI). Protocols for Determination of Limits of Detection and limits of Quantitation; Approved guideline – CLSI document EP17-A (ISBN 1-56238-551-8) CLSI, 940 West Valley Road, Suite 1400 , Wayne , Pennsylvania 19087-1898 USA, 2004.

7. Clinical Laboratory Standards Institute (CLSI). Interference Testing In Clinical Chemistry Approved Guidelines – Second Edition. CLSI document EP7-A2 (ISBN 1-56238-000-0) CLSI, 940 West Valley Road, Suite 1400 , Wayne , Pennsylvania 19087-1898 USA, 2005.

8. Clinical Laboratory Standards Institute (CLSI). User Verification Of Performance For Precision And Trueness Approved Guidelines – Second Edition. CLSI document EP15-A2 (ISBN 1-56238-000-0) CLSI, 940 West Valley Road, Suite 1400, Wayne , Pennsylvania 19087-1898 USA, 2005.

DRAFT

9. Clinical and Laboratory Standards Institute (CLSI). User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline. CLSI document EP12-A (ISBN 1-56238-468-6). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2002.

DRAFT