At the March 7 meeting the Microbiology Devices Advisory Panel will be asked to recommend the classification of certain products that were used before 1976 (preamendments) and were overlooked at the time other preamendments products were classified, back in the early 1980s. At this meeting the panel will not be asked to discuss any particular product or submission. The panel will be asked to discuss and make recommendations on the classification of a category of products, i.e., in vitro diagnostic products for the identification of B. anthracis and Y. pestis that were available before 1976. The classification (Class I, II, or III) will be based on the review by the panel of the old devices’ safety and effectiveness. These products, when classified, will serve as predicate devices for new products with similar intended uses that do not raise new questions of safety and effectiveness.

Factors taken into consideration to classify a device include: the population for whom the device is intended, conditions of use, risk/benefit assessment, reliability of the devices based on valid scientific evidence, and level and types of controls (general or special) that can offset risks. FDA is providing you with information about the preamendments B. anthracis and Y. pestis in vitro diagnostic products that have been identified. This information includes the history of the products, how they were used, factors known to affect the use of these products, and types of controls that are likely to minimize the risks associated with their use. FDA has also included proposed language for the classification regulation describing these products.

GENERAL BACKGROUND

Bacillus anthracis and Yersinia pestis are bacterial pathogens that cause disease primarily in animals, and also in humans in contact with infected animals. Vaccination of animals and high-risk workers had virtually eliminated human anthrax by the 1970s and human anthrax was rarely reported in the U.S. before October 2001. Natural cases of human plague are rare (averaging 8/year) and limited to rural isolated areas of the southwest, and along the Pacific Coast. Case incidence has increased since 1975 possibly due to increased transmission to humans as residential areas encroach upon formerly rural areas, and increased transmission by domestic cats. Both agents are on the “Critical Biological Agents” list as agents likely to be used as biological weapons.

Products for the laboratory diagnosis of these organisms were not classified with other preamendments products. Several products were available preamendments although not widely used in many clinical laboratories in the U.S. These products were used extensively for surveillance and control activities and for identifying agents in specimens from animals.

FDA has identified specific bacteriophages and certain antibody and antigen reagents as preamendments. Please refer to the attached summaries and key publications for information on these products.
RISKS ASSOCIATED WITH THE USE OF THE OLD DEVICES

The risks that are specifically associated with the use of preamendments bacteriophages, antibody and antigen reagents for the identification of *B. anthracis* and *Y. pestis* are those associated with:

- Laboratory errors (including incorrect procedures, misinterpretation of results, inappropriate use) that can cause false negative and false positive results;
- Functional reliability of the test reagents, including their expected performance, quality and integrity; and
- Biosafety and risks to laboratory workers with handling cultures and control materials.

The diagnosis of anthrax and plague are both made on the basis of clinical findings, history of exposure, and identification of the organism by the clinical laboratory. Failure to identify *B. anthracis* or *Y. pestis* from a culture isolate or directly from human specimens may delay the diagnosis and treatment of anthrax or plague (false negative). A false negative result may delay recognition of the infection and the investigation of a common source that can affect others, and in the case of plague, delay identification of patient contacts. A false positive result may delay the diagnosis of the patient’s true condition and lead to inappropriate or unnecessary treatment. A false positive result could also lead to unnecessary public health activities.

False negative or positive test results can be caused by inability of the test to characterize all strains of the organism, cross-reactivity with other species (product performance), or user error (from improper procedures or interpretation of test results) in the laboratory. Preparation or manufacturing of products may also affect performance of laboratory testing. Although use of the appropriate test control materials in the laboratory may identify problems with product manufacturing, laboratory quality control testing may not always be adequate or feasible to recognize manufacturing problems.

The quality control materials used by the laboratory will necessarily be limited by restrictions on maintaining these organisms and the rarity of appropriate specimen materials. Because handling these organisms poses a risk to laboratory workers, use of these products and the needed laboratory control materials would be restricted to laboratories with the appropriate biosafety facilities and training.
CONTROLS

During the classification proceedings, the panel may recommend special controls, if general product controls (registration/device listing, good manufacturing practices, premarket notification, records and reports) are not considered sufficient. Special controls include, but are not limited to: postmarket surveillance, performance standards, testing guidelines, and device tracking. The following controls may be applicable to these products:

- The product labeling includes indications, warnings and precautions. The warnings and precautions listed in the product labeling are written to make the user aware of potential risks associated with the use of the device, and to minimize the impact of these risks in case they occur.
- CDC has developed and disseminated organism-specific practice guides. They provide guidance for specimen collection, culture methods, and identification criteria. They also give specific instructions for transferring isolates and specimens, and for notifying local and state health departments. CDC also provides laboratory training and proficiency programs. These documents are available at the ASM website (http://www.asmusa.org/pcsrm/biodetection.htm)
- Laboratories are required to notify Departments of Health when \( B. \) \( anthracis \) and \( Y. \) \( pestis \) are suspected and to refer suspicious isolates to expert laboratories within the national Laboratory Response Network.
- Biosafety guidelines and restrictions for use in laboratories with adequate facilities, and training and experience with the identification of unusual pathogens.
- Appropriate manufacturing controls such as pre-release testing can reduce risks associated with false negative and false positive results (described above).
**Bacillus species** Preamendments reagents

**Background**

*Bacillus anthracis* is one of the oldest known pathogens, causing anthrax disease in humans and mammals, especially grazing herbivores. Humans can be infected accidentally through contact with infected animals or their products. Human cases, primarily cutaneous, are not uncommon in parts of the world where *B. anthracis* is highly endemic and animal anthrax vaccine is not widely used (Iran, Iraq, Turkey, Pakistan and sub-Saharan Africa). Remaining endemic areas in the U.S. (e.g., Texas, Oklahoma) closely parallel cattle trails of the 1800s. In the 1950s and 1960s the majority of cases were related to the manufacture of textiles in which imported fibers were used.

Decreased use of imported contaminated fibers and immunization of textile workers dramatically reduced human cases in the U.S. Anthrax is now rare in the U.S., with only three cases reported from 1984-1993. *B. anthracis* is the bacterial agent believed most likely to be used as a biological weapon and the first deaths since 1975 occurred in 2001 from criminal dissemination.

*Bacillus cereus* causes gastrointestinal disease that is generally self-limiting. B. cereus can also rarely cause nongastrointestinal infections, particularly with IVDU and immunosuppressed individuals. Rare nongastrointestinal infections are associated with trauma or surgery, particularly implants, catheters and shunts. Other Bacillus species are occasionally reported to cause gastrointestinal and nongastrointestinal disease. The probability of incorrect species identification confounds many of these reports.

*Bacillus* species are ubiquitous in nature and persist under adverse conditions due to resistance of their spores. As a result, Bacillus species are frequently encountered in clinical laboratories as “contaminants,” but also as significant pathogens. Cultured specimens from which Bacilli may be recovered include blood, CSF, other sterile body fluids, sputum, skin lesions, and biopsy material.

Differentiation between *Bacillus* species isolates in the laboratory can be difficult. For clinical purposes, the differentiation of *B. anthracis* from *B. cereus* is most important, for clinical diagnosis and for recognizing need for enhanced biosafety measures. A large number of phenotypic tests can be used, but often only a single one separates different species. The principal distinguishing characteristics between *B. cereus* and *B. anthracis* are motility, hemolysis on blood agar, and gamma phage susceptibility. No one characteristic is sufficient to discriminate the different species, since *B. cereus* can be nonmotile, *B. anthracis* can be weakly hemolytic, and rare strains other than *B. anthracis* can be susceptible to lysis by gamma bacteriophage.

Preamendments reagents for the laboratory differentiation of *Bacillus anthracis* from other *Bacillus* species include antibodies specific to organism capsule or other cellular components, organism-specific bacteriophages, and antigens for serological detection of antibodies to capsular, somatic, or other antigens.
Bacteriophage gamma

Description: a specific bacterial virus used in a culture plating method to distinguish *B. anthracis* from *B. cereus* and other *Bacillus* species. The bacteriophage can rarely lyse other *Bacillus* sp. and can fail to lyse rare strains of *B. anthracis*.

In 1951, McCloy reported isolation of a bacteriophage that lysed strains of *B. anthracis*, the parent *B. cereus* strain W, 2 of 56 other *B. cereus* strains and none of 18 other *Bacillus* species. Brown and Cherry (1955) subsequently isolated gamma-phage from the original W bacteriophage of McCloy. Brown et al showed (1958) the phage specifically lysed vegetative *B. anthracis* (122 strains) and not *B. cereus* strains tested (115 strains); 8 of 70 *B. cereus* var. *mycoides* (all dissociant strains) were lysed.

Buck et al (1963) reported that 63 of 74 (85%) *B. anthracis* strains and a strain of *B. megaterium* out of 64 other species tested were lysed by the gamma-phage. USAMRIID recently validated performance showing that only 2 known phage-refractory *B. anthracis* strains were not lysed (out of 50 tested) and that a rare *B. cereus* and *B. mycoides* out of 50 non-*B. anthracis* species were lysed [see Abshire et al, 2001. ASM poster].

The following are reported factors that may contribute to false positive or negative results with the gamma phage testing:

- Differences in behavior of variant phage strains
- Media used may affect visualization of lysis
- Extended or shortened incubation
- Altered phage titer and stability
- Heavy inocula
- Laboratory and technologist inexperience interpreting partial lysis
- Not using phage results n conjunction with other microbiological observations (growth characteristics, Gram reaction, catalase, hemolysis and motility)

Fluorescent antibody detection of *B. anthracis* capsule

Description: A fluorescein-labeled antibody against *B. anthracis* capsule is used to microscopically visualize specific binding with cultured bacteria. The test can be performed with culture growth or can be done on specimens that have Gram positive bacteria resembling *B. anthracis*. Fluorescence with capsules is presumptive evidence for identification of *B. anthracis* that must be confirmed with other testing.

Cultured isolates of *B. anthracis* produce capsules when grown on bicarbonate agar with serum in the presence of CO₂. Vegetative organisms in specimens from patients or animals infected with *B. anthracis* are also usually encapsulated. Virulent *B. anthracis* strains can lose ability to encapsulate when recovered from environmental sources or maintained in laboratory culture media.

The polypeptide-poly-D-glutamic acid capsule of *B. anthracis* can be visualized by certain stains (M’Fadyean, Giemsa, India ink, and toluidine blue have been described).
Cherry & Freeman (1959) reported using immunofluorescence to identify *B. anthracis*. *B. subtilis* and *B. megaterium* also produce similar capsules that are positive by the immunofluorescent antisera prepared against encapsulated *B. anthracis*.

The following are reported factors that may contribute to false positive or negative results with fluorescent antibody testing:

- Capsular and cell wall antigens of *B. anthracis* are shared by other species
- Preparing high titer antiserum in animals is difficult and a safety concern
- Spore surface antigens are not species-specific
- Growth conditions affect encapsulation

**NOTE:** Hyperimmune antiserum prepared in rabbits against either live virulent bacteria or formalin-killed virulent bacteria was commonly used for the Ascoli precipitin test. This test was first described in 1911. The procedure is primarily used for detecting *B. anthracis* antigens in tissues of animals suspected to be infected. It was also used to test hides and other animal products for evidence of anthrax contamination. The test is not highly specific but was useful in that other *Bacillus* species would not likely have proliferated and deposited precipitating antigens as would *B. anthracis*.

*B. anthracis* antigens for antibody detection

Antibody detection can be used for confirmation of anthrax if the patient survives, and early antibiotic treatment does not abrogate antibody expression. Such serological testing is most useful for monitoring responses to anthrax vaccines and for epidemiological investigations. Whole cell antigen preparations are not specific to *B. anthracis*. Protective antigen was first identified by Gladstone (1946) in cell-free culture filtrates.

A skin test preparation (Anthraxin) has been used since 1962 in the former USSR. Anthraxin also contains antigens that are not highly specific for *B. anthracis*.

FDA is continuing to look for information relevant to the classification of antigen products. If these become available they will be forwarded to you at a later date.

**References provided:**


Yersinia pestis Preamendments reagents

Background

Yersinia pestis causes disease in humans and animals. Plague is primarily a zoonotic disease with reservoirs worldwide. It has been described since Biblical times. In the US, most cases are contracted via fleabites from infected rural wild rodents (squirrels, mice, rabbits, prairie dogs) and more recently domestic cats. Enzootic disease is limited to areas west of the Rocky Mountains.

Naturally-occurring human disease is not common (390 cases were reported in the US from 1947 to 1996), and is rarely seen outside enzootic areas except for travelers. Case incidence has increased since 1975 possibly due to increased transmission as residential areas encroach upon formerly rural areas, and increased transmission by domestic cats. Y. pestis is also on the list of “Critical Biological Agents” as having potential to be used as a biological weapon.

Naturally acquired infections are primarily bubonic or septicemic with some developing pneumonia secondary to the initial route of infection. Primary pneumonic plague results from aerosol transmission between an infected human or animal with plague (2% of the 390 cases from 1947-1996). Pneumonic plague has occurred from cat-transmitted disease. Intentional dissemination of plague would most probably occur by aerosolization and cause primary pneumonic plague. Plague is fatal in 50% of infected humans, if untreated. Septicemic and pneumonic plague are clinically indistinguishable from other syndromes.

Reagents and methods for bacteriologic diagnosis of Y. pestis are referred to in the medical literature, including pitfalls of a presumptive identification [see Bibel & Chen, 1976]. A serologic response may take 10 to 14 days to develop and can provide retrospective evidence of infection. In the 1970s when preamendments products were first classified, clinical laboratories throughout the U.S. did not encounter the organism often and specific reagents were mostly used at certain public health laboratories and reference laboratories. Classical staining with Gram, Giemsa, Wright, or Wayson stains can suggest Y. pestis, but is nonspecific as other bacteria can have the “safety-pin” appearance, and not uncommonly Y. pestis will not have this appearance. Fluorescent antibody against Fraction 1 capsular antigen directly on clinical specimens can be presumptive evidence when clinical history and presentation is suggestive.

Specimens collected for culture include blood, bubo aspirates, sputum, CSF or skin lesion scrapings. The organism is identified from culture growth by morphology, preferential growth at 27-28°C, biochemical tests, fluorescent antibody testing, and susceptibility to a specific bacteriophage. Rapid biochemical identification methods are generally not useful and may cause erroneous results, as the organism is slow growing and relatively inactive biochemically. Automated microbiological test systems may not be able to differentiate Y. pestis from other similar Gram-negative bacteria, most notably Y. pseudotuberculosis, a closely related enteric pathogen commonly found in environmental samples.
FDA has identified three products that were overlooked when pre-1976 products were first classified. These products are used to identify *Y. pestis* or to provide serological evidence of infection with *Y. pestis*. The following sections describe the preamendments history of these three products, their performance characteristics, and factors associated with inaccurate results.

**Bacteriophage**

*Description:* A specific bacterial virus used in a culture plating method to distinguish *Y. pestis* from *Y. pseudotuberculosis*. The procedure is performed at 20-25°C, as the bacteriophage can lyse *Y. pseudotuberculosis* at 37°C, but not at the lower temperature. Lysis at 22-25°C provides presumptive evidence that a culture isolate is *Y. pestis*.

In 1950, the WHO Expert Committee of Plague recommended using a specific bacteriophage for identification of *Y. pestis* (*P. pestis* at that time). Cavanaugh and Quan described a method for using Phage H lyophilized on filter paper strips (Rapid identification of *P. pestis* [1953, AJClinPathol 13:619-620]. CDC and public health laboratories used this method for many years. The preparation of the gamma phage reagent is well described [Bahmanyar & Cavanaugh, 1976. Plague Manual. WHO].

Test methods that have been described, assess specific lysis at both temperatures (room temperature and 37°C) and compare it to that of control organisms.

The following are reported factors that may contribute to false positive or negative results:

- Differences in behavior of variant phage strains
- Incubation temperatures critical for correct results
- Media used may affect visualization of lysis
- Mixed cultures may overgrow and mask lysis
- Altered phage titer and stability
- Laboratory and technologist inexperience
- Not using in conjunction with other microbiological observations (growth characteristics, Gram reaction, growth at 28°C, catalase, oxidase and urease, DFA)

**Fluorescent Antibody Reagent**

*Description:* A fluorescein-labeled antibody against Fraction 1-antigen that is used to microscopically visualize specific binding with cultured bacteria. The test can be performed with culture growth or can be done on specimens which have Gram negative bacteria resembling *Y. pestis*. Presence of F1 antigen is presumptive evidence of *Y. pestis* that must be confirmed with other testing.

Lederle Laboratories, Inc. prepared rabbit anti-plague serum against whole *Y. pestis* cells for testing done by WRAIR. WHO described a method for preparing anti-F1 serum used in a direct immunofluorescence assay.
Antigen expression is optimal when cultures are grown from 33-37° C. No cross-reactivity is seen with Y. pseudotuberculosis or E. coli.

The following are reported factors that may contribute to false positive or negative results:

- Although specific for F1, fluorescence with FA reagents is not definitive for Y. pestis
- Expression of F1 antigen by different strains may vary
- F1 antigen can be lost with storage
- Organism density of prepared slides can affect interpretation (antigen overload)

**Fraction 1 Antigen for HA Tests**

*Description:* Fraction 1 purified antigen is a protein with antiphagocytic properties from the capsular envelope of Y. pestis. It is highly immunogenic in both human and mammalian hosts. This reagent is used to sensitize sheep erythrocytes for hemagglutination (HA) testing to detect antibody responses to F1 in human or other host sera. Significant levels of human antibody to this antigen can be retrospective confirmation of Y. pestis infection or can be presumptive when a single serum sample is tested.

The Fraction1 envelope antigen of *Y. pestis* was first isolated in 1947 by Baker et al; protein and glycoprotein components were identified and characterized. This antigen was used for detecting specific antibody to *Y. pestis* in complement-fixation (CF) and passive hemagglutination (PHA) tests developed by Chen et al (1952) and Chen & Meyer (1954). The indirect hemagglutination WHO standard method (described in 1965 by Cavanaugh et al), was used extensively for serological surveys. These tests were useful when *Y. pestis* could not be recovered from tissues, bubo fluid or blood. They were also used in early plague immunization studies.

In an evaluation with sera from 83 human plague patients, 78% were positive by serological tests, while only 36% were bacteriologically positive (Meyer, 1964). The passive HA (PHA) test was found to be 20-50 times more sensitive than the CF test. No cross-reactivity between *Y pestis* and *Y. pseudotuberculosis* was found with either CF or PHA tests.

In 1972, Marshall et al published findings from testing 307 immunized and 75 nonimmunized individuals using agar-gel precipitin inhibition test, CF and PHA. PHA was the most sensitive. The test is generally reproducible within 2-fold dilutions. HA titers persisted for at least a year in a pneumonic plague patient studied. The HA test was found to be more reliable than the CF test for a variety of wild and domestic mammals. Again, Marshal et al showed utility of hemagglutination testing for evaluating titers of vaccinees. In this report, 572/576 sera had titers within one dilution when retested after 7 months [Marshal et al, 1974. Plague Immunization III. Serologic response to multiple inoculations of vaccine. J of Inf Dis 129:S26-S29].

The soluble F1 antigen in physiological saline is stable at 4° C for at least 5 years. Purity and concentration of F1 was found to correlate with the degree of specificity. Methods for preparation are published [Chen & Meyer, 1966; Rust et al, 1972].
In the 1970’s, F1-sensitized sheep red cells were available from Walter Reed Army Institute of Research [Manual of Clinical Immunology, 1976].

The following are factors contributing to false positive or negative results with the HA testing:

- Impure antigen preparations
- Incorrect concentration of F1 preparation
- Serum sample obtained too early (serological response may appear as early as day 5 and peaks around day 14, persisting at least to day 40)
- Occasional infections with nonencapsulated Y. pestis
- Prozone effects
- Inability to differentiate between recent and past infection
- Subjectivity of endpoints
- Nonspecific reactivity of heterophiles

References provided:


