This guidance was written prior to the February 27, 1997 implementation of FDA's Good Guidance Practices, GGP's. It does not create or confer rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both. This guidance will be updated in the next revision to include the standard elements of GGP's.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Memorandum

Date May 31, 1990

From Chief, Hematology, Pathology, and Microbiology Branch, Division of Clinical Laboratory Devices, Office of Device Evaluation, Center for Devices and Radiological Health

Subject Review Criteria for Devices Assisting in the Diagnosis of C. Difficile Associated Disease

To Interested Manufacturers

We have developed a draft document entitled, "Review Criteria for Assessment of Laboratory Tests Directed at Assisting in the Diagnosis of C. Difficile Associated Disease." Since the document lists items we will be reviewing, it is intended to assist manufacturers in the preparation of marketing submissions for these types of devices.

Since this area of in vitro diagnostics is rapidly expanding in the clinical laboratory, we are soliciting your ideas, recommendations, and comments regarding the attached review criteria. We will appreciate receiving your comments so that we can incorporate as many improvements as possible in a revision. The availability of the document will be made in the FEDERAL REGISTER. Please address comments to:

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Attachment

Version Original

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This is a flexible document which represents the current major concerns and issues regarding in vitro diagnostic devices as based on recent scientific and clinical experience and on traditional submissions by manufacturers. As advancements are made in science and medicine, these review criteria will be evaluated and revised as necessary to accomodate new knowledge.

REVIEW CRITERIA FOR ASSESSMENT OF LABORATORY TESTS DIRECTED AT ASSISTING IN THE DIAGNOSIS OF *C. DIFFICILE* ASSOCIATED DISEASE

DEFINITION: The generic type device is intended for use in clinical laboratories to aid in the diagnosis of *C. difficile* associated disease.

PURPOSE: The purpose of this document is to ensure reliable and reproducible commercially available tests for detecting *C. difficile* organisms and/or its products in fecal specimens.

This document also provides guidance as to what information should be presented by a manufacturer for the Food and Drug Administration's (FDA) consideration before one of these devices may be cleared for marketing. The information enables FDA to make well informed decisions based on a uniform data base.

I. Background

Currently there is no laboratory test that defines the presence of *C. difficile* associated gastrointestinal disease (CDAD). This organism is associated with a spectrum of gastrointestinal conditions, ranging from uncomplicated carrier state to antibiotic-associated diarrhea (AAD) to antibiotic-associated colitis (AAC) or pseudomembranous colitis (PMC), the most severe life-threatening form. Therefore, these devices serve as an "aid in the diagnosis of *C. difficile* associated disease". Historically, the two tests which have been used for this purpose are culture and identification of the organism from stool, and cytotoxicity assays in tissue cell cultures for the detection of toxins or virulence factors.

Key issues in the review of these devices have centered on specific intended use statements dependent on the type of device manufactured and what specifically that device claims to detect. This should be clearly stated by the manufacturer in the product insert with emphasis on analyte detected such as (1) organism, specific antigenic components(s), (2) toxins, and/or (3) Toxin A or (4) Toxin B, etc. The intended use determines the appropriate device, culture methodology, or "gold standard" to which the new device must be compared for determination of substantial equivalence.

The laboratory tests for the presence of C. difficile, its antigen(s) or the toxin(s) in stool or stool supernates include:

1. Culture and biochemical identification of the organism which has been the traditional means for detecting the presence of the organism in stool.

2. Detection of toxin(s) which has traditionally utilized cytotoxicity assays in tissue cell cultures with neutralization by C. sordelli antisera, C. difficile antisera or specific antisera to C. difficile Toxin A and/or Toxin B.

Toxin A is an extracellular enterotoxin responsible for most of the pathogenic effects in the human gut. It causes specific effects when injected into the ileal loop of the rabbit.

Toxin B is an extracellular cytotoxin responsible for in vitro cytopathic effect (CPE) in tissue cell culture or cytotoxicity assays. The in vivo effects of Toxin B are unknown; however, it is felt that it may act synergistically with Toxin A to cause disease in humans.

The "Gold Standards" (to be used at the clinical test sites) are as follows:

1. Devices designed to indicate the presence of the organism should be compared to culture of the stool for isolation and subsequent identification of C. difficile isolates.

2. Devices designed for toxin detection (whether the device detects toxin A or toxin B) should determine (a) the cytopathic effect of the fecal supernate in tissue culture and (b) evaluates the neutralization of that effect with anti-toxin (see above). This approach assumes that toxigenic strains produce both A and B toxins.

Additional data may be necessary to substantiate claims of intended use and determination of substantial equivalence based on similarities or differences between devices, detecting reagents, levels of detection, relevant clinical performance data, etc.

Careful consideration should be paid to the appropriateness of product labeling as it relates to its aid in the diagnosis of CDAD, clinical relevance of testing, appropriate presentation of performance data, and limitations of testing.

II. Device Description

Each manufacturer must specify in the package labeling:

A. The specific technology/methodology upon which the device is based,

B. The intended use based on the technology/methodology employed in the device,

C. The specific analyte detected, and

D. That these devices "aid in the diagnosis" of *C. difficile* associated disease (CDAD).

The types of devices FDA reviews are based on the following:

1. Organism - devices directed at detection of the *C. difficile* organism or specific structural antigenic components of the organism.

2. Toxins - devices that detect Toxin A or Toxin B, or both.

   (Virulence Factors - devices that detect virulence factors other than toxin responsible for *in vivo* pathologic effect are not the subject of these review criteria).

III. Performance Considerations

The concern of the Food and Drug Administration (FDA) differs as to the type and volume of data to be submitted by a manufacturer in support of an application for marketing a device that detects the presence of *C. difficile* or its toxins in stool. These concerns are summarized:

A. Organism. Requires submission of Premarket Notification (510(k)).

   Predicate device - *C. difficile* culture and identification of organism.

B. Specific Toxins (A or B or both). Requires submission of Premarket Notification (510(k)).

   Predicate device - Tissue culture cytotoxicity assay with confirmatory anti-toxin neutralization, specific tests for toxin A.

C. Virulence factors. Not subject of these review criteria.

IV. Specific Performance Considerations

For the specific information and data needed to make a determination of substantial equivalency and in order to facilitate a scientific review, the following Critical Performance Criteria must be presented.

A. In-house Studies

o Devices directed at detection of organism or specific structural antigenic components(s) of the organism.

For data developed in-house - submission documents should contain the following information

1. What is specifically being detected?

2. How is it being detected? (method such as latex, ELISA, etc.)

a. characterize the detecting reagent such as monoclonal antibody, polyclonal antibody, probe, etc.

(1) How is it prepared and purified?

(2) Level or limits of detection of the target (analyte), i.e., Cfu's, ng of protein, etc.

(3) Does it cross react with other organisms found as part of the normal human colonic flora (list organisms tested)?

(4) Does it react with well-characterized toxigenic and non-toxigenic C. difficile reference strains as well as recent clinical isolates?

(5) Are there any known interfering substances? (false positive, false negative, equivocal results)?

(6) Do equivocal results occur? Why?

(7) Reproducibility within and between assays.

(8) Literature cited to support claims.

3. Describe how the performance of the device is affected by various storage conditions of the stool specimen?

o Devices directed at toxin A or B or both

Data developed in-house - submission documents should contain the following information

1. What is specifically being detected?

2. How is it detected (method), i.e., ELISA, tissue culture cytotoxicity, CIE, other?

   a. Characterize the detecting agent such as monoclonal, polyclonal antibody, other.

      (1) How is it prepared and purified?

      (2) Level or limits of detection of the target analyte, i.e., ng protein, etc.

      (3) Is there cross-reactivity to other human colonic flora organisms that produce similar toxin(s)?

      (4) Are there any known interfering (false positive, false negative) substances?

      (5) Do equivocal results occur? Why?

      (6) Results with reference toxigenic and non toxigenic strains of C. difficile as well as recent clinical isolates.

      (7) What is the stability of stool specimens with known detectable factor stored at various temperatures and tested periodically (also addressed in labeling)?

      (8) Reproducibility within and between assays.

      (9) Literature cited to support claims as well as recent clinical isolates.

3. How is the performance of the device affected by various storage conditions of the specimen?

B. Clinical Study (testing should be performed in at least 2 geographically separated outside laboratories experienced in testing for CDAD).

- o Devices directed at detection of organism or specific structural antigenic component(s) of the organism.

  1. Specimen acceptability - only patient fecal specimens submitted to the testing laboratory to rule out suspected Clostridium difficile associated disease or the presence of the organism as part of the patient's normal flora.

     a. Diarrheal stool - recommended for the acute disease

        (1) From patients with a history of antibiotic therapy within last 8 weeks or

        (2) Patients with no history of antibiotic therapy

     b. Formed stool to rule out carrier state in the following circumstances:

        (1) as part of normal flora,

        (2) because of nosocomial acquisition,

        (3) or post therapy for CDAD (persistence of organism)

  2. Procedure

     a. Identify name of test site, principal investigator, and who performed clinical testing at the site.

        (1) Provide description of culture and identification methods

        (2) Ideally specimen should be cultured and device tested at the same time.

        (3) All tests should be performed within two hours of collection or receipt in the laboratory, unless can show that results are not affected by delay (support with data developed in-house).

        (4) Repeat testing of previously positive patient specimens should be excluded unless submitted to rule out carrier state post RX for CDAD

        (5) Repeat testing of previously negative patient specimens should be encouraged particularly if diarrhea persists.

(6) A minimum of 100 positive culture results from all clinical sites, preferably equally distributed positives from each of the sites.

o Devices Directed At Toxin (A or B or both)

1. Specimen acceptability - only diarrheal stool submitted to rule out CDAD.

2. Procedure

a. Name of clinical test site and principal investigator and who performed clinical testing.

(1) Provide description of cytotoxin assay (toxin B) and neutralization studies or other method (toxin A) used at site.

(2) Both device and reference method testing should be done within same time frame (within hours of each other). Specimens should not be frozen then tested unless both tests are done after freezing or in house data demonstrates that freezing specimen will not interfere with test.

(3) Repeat testing of previously negative patient specimens should be encouraged particularly if diarrhea persists.

(4) A minimum of 100 positive results from all clinical sites (preferably equally distributed positives from each of the sites).

C. Assessement of results developed in-house and at clinical sites.

1. Data developed in-house - satisfactory documentation of all items

2. Data developed at clinical test sites

(a) Culture or toxin results vs. device

(1) may include comparison to similiar competitor device

(2) Calculate sensitivity and specificity to "gold standard".

(b) Explanation for each discrepant result.

V. Labeling Considerations

Labeling should conform to 21 CFR 809.10 requirements, in order to make a substantial equivalence determination. The following information should be included in the package insert.

A. Intended Use

Product Inserts - Labeled as "an aid" in the diagnosis of C. difficile associated disease.

B. Limitations of test

1. Specimen type, integrity (time period and conditions for storage)

2. Level of detection

The test does not define the presence of disease. The demonstration of the organism, or toxin(s) of C. difficile in diarrheal stool may or may not correlate well with clinical findings of colitis.

3. The test does not define the presence of CDAD, but only demonstrates the presence of organism or toxin(s) in stools. These test results should be interpreted by a physician in conjunction with other laboratory test results and patient clinical findings.

C. Performance characteristics

1. concordance, specificity, sensitivity

a. organism detection vs culture with or without competitor device

b. toxin vs toxin not organism vs toxin.

D. References

Produce inserts should be reflective of the current understanding of C. difficile associated disease with appropriate citations. The enclosed citations are not designed to be inclusive or exclusive.

1. Gerding, D.N., Disease associated with Clostridium difficile infection. 1989. Annals Int. Med. 110:255-257.

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3. Fekety, R. Antibiotic-associated colitis. In: Principle and Practice of Infectious Diseases. 3rd Edition. 1990. Ed. Mandell, Douglas, Bennett. 863-869.

4. Peterson, L.R., M. M. Olson, C. J. Shanholtzer and D. N. Gelding. Results of a prospective, 18 month clinical evaluation of culture, cytotoxin testing, and culturette brand (CDT) latex testing in the diagnosis of Clostridium difficile-associated diarrhea. 1988. Diag. Microbiol. Infect. Dis. 10:85-91.

5. Bartlett, J.G. Clostridium difficile: Clinical Considerations. 1990. Rev. Infect. Dis. 12, S243-251.