

LABORATORY PROCEDURE

CULTURETTE® BRAND CDT® CLOSTRIDIUM DIFFICILE TEST

I. INTENDED USE

The CULTURETTE® BRAND CDT® test is a rapid latex agglutination test intended to assist in the diagnosis of *Clostridium difficile*-associated disease for the detection of *Clostridium difficile* antigens in stool specimens.

II. SUMMARY AND EXPLANATION

C. difficile is an important cause of antibiotic-associated diarrhea that in its most serious form can result in pseudomembranous colitis and death. Rapid intervention in patients with diarrhea may prevent progression to advanced stages of *C. difficile*-associated disease and their complications. *C. difficile*-associated disease, which includes antibiotic-associated diarrhea (AAD), colitis (AAC), and pseudomembranous colitis (AAPMC), is a complication of treatment with a wide variety of antibiotics. Cytotoxins produced by *C. difficile* mediate the clinical syndrome.^{1,2} Nosocomial transmission and environmental contamination within the hospital have been established.³ The diagnosis of AAPMC has depended upon the identification of the characteristic lesions of AAPMC as seen by sigmoidoscopic or colonoscopic exam and/or the demonstration of *C. difficile* and/or its toxins in stool specimens. The complexity of culture and toxin assay procedures led to the development of the CULTURETTE BRAND CDT rapid latex agglutination test for *C. difficile* antigens.

Comparisons of the results of latex agglutination testing, culture, toxin assays and clinical evidence have shown correlation but not complete diagnostic agreement.^{4,9} Clinical signs and symptoms of disease such as the duration of antibiotic treatment, the duration and severity of diarrhea and the presence of colitis or pseudomembranes are all factors that must be considered when diagnosing AAPMC. The rapid result provided by the CULTURETTE BRAND CDT latex agglutination test makes it an excellent choice in defining an etiologic role for *C. difficile* in a patient with diarrhea.¹⁰

III. PRINCIPLES OF THE PROCEDURE

The CULTURETTE BRAND CDT *C. difficile* test is a rapid latex agglutination test which detects *C. difficile* antigens in stool specimens. The antigens are associated with strains of *C. difficile* that are actively producing toxin and with strains that are not.

Latex beads coated with antibody react with *C. difficile*-associated antigens in the stool supernatant to form a lattice of visibly agglutinated particles. The antigens are soluble and are found in the supernatant solution following centrifugation with specimen dilution buffer. The sensitized latex particles agglutinate to allow rapid visualization of aggregates following incubation with *C. difficile* antigens contained in the fecal specimen. When these antigens are absent, or below detectable limits, the latex particles do not agglutinate but remain as a smooth evenly dispersed suspension. A positive control is provided to assure reactivity of the antibody-coated latex detection reagent. A negative control containing latex coated with globulin from unimmunized rabbits is provided to aid in the detection of nonspecific agglutination.

IV. REAGENTS

Reagent 1	(1.5 ml)	CDT Latex Detection , rabbit anti- <i>C. difficile</i> antigen-coated latex particles,
Reagent 2	(0.8 ml)	CDT Latex negative control , normal rabbit antibody-coated latex particles,
Reagent 3	(1.2 ml)	CDT Positive Control , <i>C. difficile</i> culture filtrate,
Reagent 4	(24.0 ml)	CDT Buffer Solution , all with 1% sodium azide (preservative).

Precautions: For In Vitro Diagnostic Use

Reagents: Do not use beyond the expiration date. Do NOT mix reagents from different kit lot numbers, or mix reagent bottle caps.

Upon removal from the refrigerator, allow reagents to warm to room temperature (15 to 30°C) before use.

Mix contents of reagent bottles by gently inverting several times before using. To assure proper drop delivery, bottles must be held vertically while dispensing free-falling drops.

Observe established precautions against microbiological hazards throughout all procedures. All specimens should be handled according to CDC/NIH(U.S. Centers for Disease Control and Prevention/National Institutes of Health) recommendations for any potentially infectious human serum, blood or other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

Warning: Reagents contain sodium azide. Very toxic by inhalation, in contact with skin, and if swallowed. Contact with acids liberates very toxic gas. After contact with skin, wash immediately with plenty of water. Sodium Azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Test Cards: Care should be taken not to finger-mark test areas, since this may result in an oily deposit and improper test results. Use each card once and discard. When spreading within confines of test areas, avoid scratching the card surface with stirrers. If the specimen does not spread to the outer perimeter of test area, use another card.

Controls: Do not use the kit if controls do not yield appropriate results.

Reading Test Results: Results should be read promptly under a high intensity incandescent lamp. Fluorescent lighting is generally insufficient to distinguish test results.

Storage: Store all reagents at 2 to 8°C when not in use. DO NOT FREEZE. Reagents should be re-capped and returned to refrigeration when not in use.

Store cards in original package in a dry area at room temperature.

V. SPECIMEN COLLECTION AND HANDLING

The fecal specimen should be collected in a container allowing easy transfer to a centrifuge tube for the extraction procedure.

The **CULTURETTE BRAND CDT** Buffer Solution must be used to prepare the stool extract. Do not use any substitutes.

It is recommended that freshly collected stool specimens be used; however, if the specimen cannot be processed immediately, it can be stored for up to 3 days at 2 to 8°C.

Freezing the stool specimen or the stool extract may result in a loss of reactivity. Tarry or fatty stools may cause nonspecific agglutination.

Specimens with excessive mucus may yield insufficient supernatant to perform the test. Sufficient material may be obtained by high speed centrifugation (15,000 x g for 15 mm), or by filtering the specimen through gauze prior to mixing with the Buffer Solution.

VI. PROCEDURE

Review "Precautions" and "Specimen Collection and Handling" prior to performing procedures. The testing area, reagents, test specimens and test components should be at room temperature (15 to 30°C) when used.

Materials Provided: All materials listed under reagents, disposable test cards and accessories.

Materials Not Provided: Centrifuge, centrifuge tubes, vortex mixer, high intensity incandescent light, rotator (90-140 rpm), wooden applicator sticks.

Also required are the necessary equipment and labware used for preparation, storage and handling of specimens.

User Quality Control: A positive control test should be performed with each patient sample. An agglutination reaction should occur within 3 min. If no agglutination reaction occurs with **Reagent 3**, the reagents should not be used.

There should be no agglutination reaction in the Negative Control circle during the 3 min test period. A Negative Control must be performed for every patient sample.

Performance of the Test:

1. MIX 0.5 g of stool (or 0.5 ml of liquid stool) with 0.5 ml of **Reagent 4** in a clean centrifuge tube.
2. VORTEX for 30 sec with a mixer.
3. CENTRIFUGE for 15 min at a minimum of 1500 x g.
4. Using a micropipet, place 1 drop of supernatant in the **TEST** circle and 1 drop in the **NEGATIVE** circle on a clean test card.
5. ADD 1 drop of **reagent 1** to the **TEST** circle and to the **POSITIVE** circle.
6. ADD 1 drop of **Reagent 2** to the **NEGATIVE** circle.
7. ADD 1 drop of **Reagent 3** to the **POSITIVE** circle.
8. MIX the contents of each circle with a separate clean applicator stick and spread over the surface of the circle.
9. ROCK the test card for EXACTLY 3 min.
NOTE: *A mechanical rotator may be used to rotate the test card for 3 min at 90-140 rpm. See "AVAILABILITY."*
10. Immediately read test results macroscopically under a high intensity incandescent light.

VII. RESULTS

Positive Test (Antigen Present) = Any test which shows more agglutination in the **TEST** than in the **NEGATIVE** circle.

Negative Test (Antigen Absent) = Any test which shows no agglutination in the **TEST** circle and in the **NEGATIVE** circle.

Nonspecific Agglutination= Any test which shows equal agglutination in both the **TEST** and **NEGATIVE** circles or agglutination in the **NEGATIVE** circle only. Retesting the specimen following an additional 1:1 dilution with buffer solution may eliminate this nonspecific agglutination reaction and allow either a positive or negative test result to be interpreted.

VIII. LIMITATIONS OF PROCEDURE

The presence or absence of *C. difficile* antigen in diarrheal stool specimens may, or may not, correlate well with clinical findings of colitis. Test results should be interpreted by a physician in conjunction with other laboratory and clinical data.

Cross-reactivity of the **CULTURETTE BRAND CDT** *C. difficile* detection reagent with proteins from *C. sporogenes* and selected strains of *C. botulinum*, *Peptostreptococcus anaerobius* and *Porphyromonas asaccharolytica* has been reported.^{4,11,12}

As with any diagnostic test, accuracy and test performance are dependent upon appropriate collection, transport, and storage of clinical specimens as well as adherence procedures described in the package insert.

IX. PERFORMANCE CHARACTERISTICS

The efficacy of the **CULTURETTE BRAND CDT** *C. difficile* test was determined in a prospective multicenter study in the United States. Stool specimens obtained from individuals with and without symptoms of antibiotic-associated diarrhea were utilized in this study. The latex test results were compared to tissue culture cytotoxicity:

		LATEX TEST		
		POSITIVE	NEGATIVE	TOTAL
CYTOTOXICITY	POSITIVE	66	9	75
	NEGATIVE	3	161	164
	TOTAL	69	170	239

SENSITIVITY of the latex test is 88.0%.

SPECIFICITY of the latex test is 98.2%.

ACCURACY of the latex test is 95.0%.

Positive Predictive Value (PPV) is 95.7%.

Negative Predictive Value (NPV) is 94.7%.

X. AVAILABILITY

Catalog #	Description
4964001	CULTURETTE® BRAND CDT® (<i>C. difficile</i>), 25 Test Kit.
4964002	CULTURETTE® BRAND CDT® Buffer, 6 x 24 ml Bottles.
49878051	Macro-Vue® Card Test Rotator (with humidifying cover), 100 rpm, automatic timer, friction drive, model 51-11(110 V).
49878054	Model 54 (220 V)
49877979	Macro-Vue Card Test Rotator Accessories Package, containing one 1 5" x 7" extension top and two humidifying covers.

XI. REFERENCES

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TECHNICAL INFORMATION: In the United States, telephone Becton and Dickinson Microbiology Systems Technical Services, toll free (800) 638-8663, Prompt 2.

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PI Rev. 11/94
Rev 10/97

