LABORATORY PROCEDURE

CULTURETTE® BRAND ***Toxin CD***

I. INTENDED USE

The **CULTURETTE®** BRAND *Toxin* **CD** Test is a rapid 70-min enzyme immunoassay (EIA) for the detection of *Clostridium difficile* toxin A (enterotoxin) in human stool. This assay is intended as an aid in the diagnosis of *C. difficile*-associated disease.

II. SUMMARY AND EXPLANATION

C. difficile is an important cause of antibiotic-associated diarrhea, which in its most serious form can result in the clinical syndrome of pseudomembranous colitis and significant mortality. Although *C. difficile* may be a part of the normal bacterial intestinal flora, it may become an opportunistic pathogen following the patient's treatment with antibiotics and subsequent alteration of the normal intestinal flora. Under the proper conditions, toxin-producing strains of *C. difficile* produce two toxins - toxin A, a tissue-damaging enterotoxin, and toxin B, an in vitro cytotoxin.[1] At the present time, the literature states that both toxin A and toxin B are produced at the same time.[2] The clinical symptoms associated with the disease are thought to be mainly due to toxin A, and to date there is not convincing evidence that toxin B has any important biologic activity in naturally-occurring disease.[3]

The most common clinical diagnostic aids for *C. difficile* antibiotic-associated colitis have been cell culture cytotoxicity (CTA) and latex agglutination (LA) assays.[4] The CTA assay detects toxin B through the cytopathic effect on cell culture. While very sensitive, this assay requires a minimum of 2 days to complete. Latex agglutination detects the antigens of *C. difficile* rather than the specific toxins, but is regarded as a valuable rapid assay in defining an etiologic role for *C. difficile* in patients with diarrhea.[5] Neither of these assays detect toxin A, the putative cause of disease.

Ill. PRINCIPLE OF PROCEDURE

The **CULTURETTE** BRAND *Toxin* **CD** Test utilizes an anti-toxin A capture antibody coated on microwells. The patient sample and an enzyme-conjugated anti-toxin A detector antibody are added to the microwells and incubated at 35 to 37°C for 1 h. If toxin A is present, a reactive anti-toxin enzyme complex develops. After washing to remove unbound conjugate, a substrate and a chromogen are added and incubated at room temperature for 10 min. Color develops in the presence of bound enzyme. The reactions are then read visually, or spectrophotometrically if desired.

IV. REAGENTS

CULTURETTE BRAND *Toxin* **CD** Kit:

Reagent 1	(20.0 ml)	Sample Buffer, protein stabilizer, with 0.02% thimerosal (preservative).
Reagent 2	(10.0 ml)	Conjugate Reagent, polyclonal (rabbit) antibody specific for *C. difficile* toxin A conjugated to horseradish peroxidase in a buffered protein stabilizer, with 0.01 % thimerosal (preservative).
Reagent 3	(225.0 ml)	Wash Reagent, nonreactive detergent in deionized water.
Reagent 4	(10.0 ml)	H_2O_2 Reagent, buffered solution containing hydrogen peroxide.
Reagent 5	(10.0 ml)	TMB Reagent, solution containing tetramethyl-benzidine.
Reagent 6	(10.0 ml)	Stop Reagent, 1.0 N sulfuric acid. *Caution*: Avoid skin contact; if contact occurs, wash immediately with water.
Control -	(1.9 ml)	Negative Control, contains protein stabilizers and 0.02% thimerosal (preservative).
Control +	(1.9 ml)	Low Positive Control, contains inactivated C. di*fficile* toxin A, protein stabilizers, and 0.01% thimerosal (preservative).
Control + +	(1.9 ml)	High Positive Control, contains inactivated *C. difficile* toxin A, protein stabilizers, and 0.01% thimerosal (preservative).

Microwell strips coated with polyclonal rabbit antibodies specific for *C. difficile* toxin A.

Precautions: For in Vitro Diagnostic Use.

After review by the Centers for Disease Control and Prevention (CDC), and the Food and Drug Administration (FDA) under CLIA '88, this product has been identified as high complexity. The CDC Analyte Identifier Code is 1022; the CDC Test System Identifier Code is 07486 (visual) and 07487 (spectrophotometric).

Reagents: Do not use beyond the expiration date. Upon removal from the refrigerator, allow reagents to warm to room temperature before use. Do NOT mix reagents from different kit lot numbers.

Avoid prolonged exposure of **Reagents 4** and **5** to strong light.

To assure proper drop size delivery, hold the dispensing bottle vertically above the microwell, dispensing one free-falling drop at a time.

Avoid skin contact with **Reagent 6** (1 N H_2S0_4). Flush with water immediately if contact occurs.

Controls: Do not use the kit if positive and negative controls do not yield appropriate results. Positive controls are made with inactivated toxin A antigen; however, they should be handled as potentially biohazardous material.

Transfer Pipets: Single use; do not reuse.

Storage of Reagents: Upon receipt , refrigerate reagents at 2 to 8°C. DO NOT FREEZE Reagents should be recapped and returned to refrigeration when not in use, taking care not to mix color-coded caps.

Reagent 3 (Wash) may be stored at room temperature (with original cap) or refrigerated at 2 to 8°C. **Allow 90 min to warm to room temperature following refrigeration.**

Microwells: Do not reuse. Perform microwell washing exactly as described under "Performance of Test". Inadequate washing may result in elevated background reading or random color development in toxin A negative samples.

The microwells are contained in a resealable foil envelope designed to protect strips from moisture. The envelope should be opened as described under "Assay Procedure." Keep microwells in the resealable foil envelope until the envelope reaches room temperature. Immediately replace all unused microwells into the resealable foil envelope containing the desiccant, reseal and return to refrigeration.

V. SPECIMEN COLLECTION AND HANDLING

Collect stool specimens into a clean air-tight container with no preservative. Testing of specimens should be performed as soon as possible upon receipt in the laboratory; however, storage for up to 48 h at 2 to 8°C is permissible. If specimens are to be tested after 48h, specimens should be frozen at -70°C immediately upon receipt in the laboratory. Although the majority of positive specimens will show little or no reduction in toxin A detected after one freeze-thaw cycle,[8] *fresh specimens are preferred.*

Allow stool specimens to warm to room temperature and mix as thoroughly as possible prior to use.

Observe established precautions against microbiological hazards throughout all procedures. All specimens should be handled according to CDC/NIH recommendations for any potentially infectious human serum, blood or other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

In keeping with good laboratory practice, wear disposable gloves throughout the assay and wash hands thoroughly afterwards.

Dispose of all materials used in performing the test by autoclaving for 60 min at 121°C or by treatment with a 0.05% solution of sodium hypochlorite (1:100 dilution of household bleach) for 30 min. *Do not autoclave materials containing sodium hypochlorite.* **WARNING**: Liquid waste containing sulfuric acid must be neutralized (e.g., with 1 N sodium bicarbonate) before addition of sodium hypochlorite.

Decontamination of the reusable microwell strip holder between assays can be accomplished by treatment with 0.05% sodium hypochlorite solution as described above.

VI. PROCEDURE

Materials Provided: All materials as listed under "Reagents", Work Station, plastic Transfer Pipets, microwell Strip Holder, strip sealer, Sample Identification Template and accessories.

Materials Not Provided: A 35 to 37°C incubator, plastic or glass test tubes (12 x 75 mm) for sample dilution, vortex mixer, EIA microwell reader capable of reading absorbance at 450 nm or 450/630 nm (optional).

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

Performance of Test: Review "Precautions" and "Specimen Collection and Handling" prior to performing procedure. The testing area, reagents, test specimens and test components should be at room termperature when used.

Assure reagents are in the proper designated wells of the work station. Remove the bottle cap from **Reagent 3** (Wash), and replace with the squirt nozzle assembly provided in the kit. After the completion of daily tests, the squirt nozzle assembly on the Wash bottle should be replaced with the original bottle cap.

Gently mix by inverting all reagents prior to use. Avoid foaming.

Specimen Preparation:

1. Add 200 µl of **Reagent 1** (Sample Buffer) to a clean 12 x 75 mm tube using a calibrated micropipettor or a transfer pipet (up to the second mark from the tip of the pipet).

2. Mix stool sample as thoroughly as possible.

a. *Liquid, loose or semi-solid stool:* Using a transfer pipet, transfer 100 µl (up to the first mark) of sample into the tube containing **Reagent 1.** Using the same transfer pipet, mix by withdrawing and expelling the tube contents 2-3 times.

While keeping the transfer pipet in the tube, vortex the tube at moderate speed for 15 sec.

b. *Solid stool:* Using a wood spatula, transfer approximately 200 mg (5-6 mm diameter, "pea" size) of stool specimen into the tube containing **Reagent 1.** Emulsify the sample and discard the wood spatula into a biohazard receptacle.

Vortex tube at moderate speed for 15 sec and place a transfer pipet into the tube.

Note: Stool samples, once diluted in **Reagent 1**, must be assayed as soon as possible.

Preparation of Microwells:

1. Open the foil envelope containing the microwells by tearing or cutting at the notches between the heat seal area and the resealable band and remove the contents.

2. Remove all of the microwell stripes from the holder using the row of buttons at the front of the workstation.

3. Break off the necessary number of microwells (one per patient, plus three controls per batch) and position them in the strip holder.

4. Return all unused microwells to the foil envelope, **reseal**, and immediately return to refrigeration.

5. Identify the location of each well using the workstation Sample Identification Template.

Assay Procedure:

1. Add 2 free falling drops of **Reagent 2 (**Conjugate) to all microwells.

2. Using the transfer pipet or a calibrated micropipettor (see "Availability"), draw up 100 ul of diluted stool (up to the first mark) and allow sample to run slowly down the side of the designated microwell. Discard the transfer pipet or micropipettor tip into a biohazard receptacle.

3. Using a separate transfer pipet or micropipettor tip for each control, draw up 100 µl of **Control + +, Control +,** and **Control -** and allow control to run slowly down the side of the designated microwells.

4. Mix the microwells by gently tapping the plate on a firm surface for 10 sec.

5. Trim the microwell strip sealer to size and press firmly atop the microwells.

6. Incubate the plate in a 35 to 37°C incubator for 60 min.
Note: Ensure that proper incubator temperature is maintained throughout the 1 hr incubation period.

7. Gently remove the microwell strip sealer and wash the microwells with **Reagent 3** (Wash) as follows:

   a. DECANT by inverting (or aspirate) microwells into a biohazard receptacle.

   b. FIRMLY SLAP the inverted plate on clean paper towels placed on a solid surface.

   c. FILL each microwell with **Reagent 3** (Wash), decant (or aspirate), and firmly slap the inverted plate on clean paper towels.

   d. REPEAT step "c" 4 additional times.

   e. After the final wash, vigorously slap the inverted plates on clean paper towels with sufficient force to remove as much excess **Reagent 3** as possible *without dislodging the strips.* Do not allow microwells to completely dry at any time.

8. Clean the underbottom of all microwells with a lint-free tissue.

9. Add 2 free-faIling drops of **Reagent 4** (H_20_2) to each microwell, followed by 2 free-falling drops of **Reagent 5** (TMB).

10. Mix the microwells by gently tapping the plate for 10 sec and incubate at room temperature for 10 min.

Reading Results:

1. If visual determinations are to be performed, read the blue color within 5 min (after the 10 min incubation period in step 10). If yellow color is preferred for visual reading, add two drops **Reagent 6** (Stop) to each microwell immediately after the 10 min incubation period in step 10, gently tap for 10 sec and read yellow color within 10 min after adding **Reagent 6.**

2. If EIA microwell reader determinations are to be performed, clean the underbottom of all microwells, zero the EIA reader on air (without microwells), and read absorbance at 450 nm or 450/630 nm within 10 min after adding **Reagent 6.**

3. After completion of tests, used microwell strips may be removed from the strip holder by pressing the holder against the row of buttons at the front of the workstation. Used microwells should be disposed of as described under "Specimen Collection and Handling."

Quality Control

The **Control +** (Low Positive) and **Control -** should be included with each batch of specimens to verify performance of the reagents and proper procedural technique. A **Control + +** (High Positive) is also provided as an optional means of verifying two levels of positive reactions. This is a particular value when results are read visually as an added assurance of procedural integrity.

Patient results should not be reported if positive and negative controls do not yield appropriate results as described in the table below.

	VISUAL	450 nm	450/630nm
Control ++	Strong color (Blue or Yellow)	>1.500	>1.455
Control +	Moderate color (Blue or Yellow)	0.300-1.000	0.250-0.900
Control -	Colorless	<0.100	<0.070

VII. INTERPRETATION OF RESULTS

The **CULTURETTE** BRAND *Toxin* **CD** Test is designed to enable visual reading of a blue color after step 10 ("Assay Procedure") or alternatively, a yellow color following the addition of **Reagent 6.** The test can also be read by an EIA Microwell Reader.

1. **Blue Visual Reading**:
   Negative = Colorless
   Positive = Blue color of **any** intensity.

2. **Yellow Visual Reading** (**Reagent 6** addition):
   Negative = Colorless
   Positive = Yellow color of **any** intensity.

3. **EIA Microwell Reader, Single Wavelength (450 nm):**
   Negative = OD_{450} <0.1
   Indeterminant = OD_{450} ≥0.1 but <0.15

Positive = $OD_{450} \geq 0.15$

4. **EIA Microwell Reader, Dual Wavelength (450/630 nm):**
Negative = $OD_{450/630} < 0.070$
Indeterminant = $OD_{450/630} \geq 0.070$ but < 0.100
Positive = $OD_{450/630} \geq 0.100$

When using an EIA microwell reader to interpret reactions, indeterminate results should be repeated. If the results are still indeterminate, a fresh patient specimen should be obtained and tested. When reading visually, reactions should be recorded as positive if color of any intensity develops. Colorless reactions are recorded as negative.

5- **Note**: Extremely strong positive reactions may produce a dark brown precipitate within 10 min after the addition of **Reagent 6**, which does not interfere with interpretation of results.

VIII. LIMITATIONS OF THE PROCEDURE

The **CULTURETTE** BRAND *Toxin* **CD** Test detects the presence of toxin A in human stool. Since the level of toxin A has not been shown to have a definitive correlation with the presence or severity of disease, the assay results should be interpreted by a physician in conjunction with other laboratory and clinical findings.

Some isolates of *Clostridium sordellii* have been shown to produce a hemorrhagic toxin (HT) which has similar biologic, physiochemical and immunochemical properties as toxin A. The HT may cross react in tests for toxin A[4]. The *C. sordellii* HT strains have not been detected in patients with antibiotic-associated diarrhea and colitis. Infants and cystic fibrosis patients may have *C. difficile* toxin present in their stool without clinical significance.[6,7]

As with other brands of tests, specimen handling is important for the maintenance of toxin A titers. If testing is delayed, freezing of samples at -70°C is recommended (see "Specimen Collection and Handling").

IX. EXPECTED VALUES

Clostridium difficile is an opportunistic pathogen that exerts its toxigenic effects when the intestinal tract has been compromised in some manner, such as by antibiotic therapy.

Therefore, patients with recent antibiotic therapy or those in chronic care situations are most often infected. Up to 1 % of healthy adults may have positive stool toxin tests on culture.[10] The rate of nosocomial infections may vary with the institution, section, housekeeping practices, and patient population.[9,10] The **CULTURETTE** BRAND *Toxin* **CD** Test has given positive rates of 3.8% to 18.5% in symptomatic patients.[11] Positive rates above or below these values may be found.

X. PERFORMANCE CHARACTERISTICS

The performance of the **CULTURETTE** BRAND *Toxin* **CD** Test was determined in prospective evaluations conducted at four major independent medical centers located in geographically distinct areas of the United States. A total of 905 fresh stool specimens from symptomatic patients with suspected *C. difficile*-associated disease that were specifically submitted for *C. difficile* cytotoxin B testing were utilized in this evaluation. The mean prevalence of *C. difficile* in patient samples tested was 13%, ranging from 4% to 20%. Cytotoxin B test results were used as the primary reference method. Discordant specimens were retested in duplicate, and if still unresolved, the **CULTURETTE** BRAND *Toxin* **CD** test result was compared to a consensus result. The consensus result was determined by reviewing the clinical diagnosis, toxigenic culture result, and other testing information when available. Results of the study are presented in Table 1 where the **CULTURETTE** BRAND *Toxin* **CD** Test is interpreted by an EIA microwell reader and in Tables 2 and 3 where interpreted by blue and yellow visual color, respectively. The **CULTURETTE** BRAND *Toxin* **CD** Test delivered performance levels of 87-92% sensitivity and 95-98% specificity depending on the reading method and reference utilized. Indeterminate results are not included in the calculations for sensitivity and specificity.

TABLE 1
Comparison of **CULTURETTE** BRAND *Toxin* **CD** Test Results Using an EIA Microwell Reader Versus Cytotoxin Test Results and Cytotoxin Results Plus Resolution of Discordants

	Cytotoxin Result		Cytotoxin Plus Resolutions of Discordants	
CULTURETTE Brand *Toxin* **CD**	Positive	Negative	Positive	Negative

Positive	99	24	110	12
Negative	17	732	9	760
Indeterminate	5	19	2	3

*Sensitivity	85%	**92%**
*Specificity	97%	**98%**
Positive Predictive Value	80%	**89%**
Negative Predictive Value	98%	**99%**
Indeterminate	3%	**<1%**
Overall Accuracy	93%	**97%**
**Total Number	896	**896**

TABLE 2

Comparison of **CULTURETTE** BRAND *Toxin* **CD** Test Results Using a Visual Blue Color Endpoint Versus Cytotoxin Test Results

	Cytotoxin Result	
CULTURETTE BRAND *Toxin* **CD**	Positive	Negative
Positive	103	21
Negative	15	727

* Sensitivity	87%
*Specificity	97%
Positive Predictive Value	83%
Negative Predictive Value	98%
Overall Accuracy	95%
***Total Number	871

TABLE 3

Comparison of **CULTURETTE** BRAND *Toxin* **CD** Test Results Using a Visual Yellow Color Endpoint Versus Cytotoxin Test Results

	Cytotoxin Result	
CULTURETTE BRAND *Toxin* **CD**	Positive	Negative
Positive	104	37
Negative	14	710

*Sensitivity	88%
*Specificity	95%
Positive Predictive Value	74%
Negative Predictive Value	98%
Overall Accuracy	93%
*** Total Number	871

*Weighted averages based on the number of positive or negative specimens tested at each trial site in relation to the number of specimens tested.

**Spectrophotometric readings were not recorded for 9 specimens

***Visual endpoint results were not recorded for 34 specimens

The reproducibility of the assay was tested using three control samples containing high,low and zero amounts of toxin A. The concordance of single and dual wavelength readings for positive and negative was 100%. The intra- and inter-assay precision in liquid samples is presented in Table 4 below.

TABLE 4: **CULTURETTE** BRAND *Toxin* **CD** Assay Precision

Sample	Mean Single	Mean Dual	%CV Intraassay Single	%CV Interassay Single	%CV Intraassay Dual	%CV Interassay Dual
Control -	0.0440	0.0075	4.34	4.77	10.6	18.67
Control +	0.6692	0.6285	7.6	10..55	8.06	11.06
Control ++	2.38	2.32	9.23	13.92	9.18	13.95

A breakdown of the stool specimen consistency encountered from the symptomatic patients in this study is presented in Table 5. The information is presented from a total specimen perspective and for positive specimens only.

TABLE 5: Stool Specimen Consistency

	Total Population (N/%)	Positive Specimens (N/%)
Liquid	100 (11%)	6 (5%)
Loose	371 (41%)	67 (55%)
Semi-solid	263 (29%)	35 (29%)
Solid	150 (17%)	11 (9%)
Not Classified	21 (2%)	3 (2%)

TABLE 6

Absorbance at 450 nm		
Microorganism	In Negative Stool	In Positive Stool
Aeromonas hydophilia ATCC 7966	0.054(-)	0.980(+)
Bacillus cereus ATCC 11778	0.053(-)	0.951(+)
Bacillus subtilis ATCC 33608	0.062(-)	0.993(+)
Bacteroides fragilis ATCC 25285	0.054(-)	0.902(+)
Candida albicans ATCC 10231	0.053(-)	0.920(+)
Clostridium botulinum ATCC 17786	0.056(-)	0.966(+)
Clostridium butyricum ATCC 8260	0.057(-)	0.904(+)
Clostridium histolyticum ATCC 19401	0.055(-)	0.959(+)
Clostridium innocuum ATCC 14501	0.054(-)	0.990(+)
Clostridium novyi ATCC 19402	0.054(-)	0.832(+)

Clostridium perfringens ATCC 13124	0.055(-)	0.984(+)
Clostridium septicum ATCC 12464	0.055(-)	1.006(+)
Clostridium sordellii VPI 9048	0.280(+)	1.124(+)
Clostridium sporogenes ATCC 3584	0.057(-)	0.858(+)
Clostridium subterminale ATCC 29748	0.058(-)	0.891(+)
Clostridium tetani ATCC 19406	0.057(-)	0.839(+)
Enterococcus faecalis ATCC 29212	0.053(-)	0.903(+)
Escherichia coli ATCC 35218	0.058(-)	0.884(+)
Escherichia coli ATCC 43889	0.057(-)	0.297(+)
Escherichia coli ATCC 43894	0.056(-)	0.312(+)
Escherichia coli ATCC 43895	0.058(-)	0.281(+)
Peptostreptococcus anaerobius ATCC 27337	0.061(-)	0.808(+)
Pseudomonas aeruginosa ATCC 14207	0.056(-)	0.835(+)
Salmonella choleraesuis ATCC 14028	0.051(-)	0.838(+)
Shigella dysenteriae ATCC 29027	0.054(-)	0.942(+)
Shigella flexnerii ATCC 12661	0.053(-)	0.941(+)
Shigella sonnei ATCC 25931	0.056(-)	0.969(+)
Staphylococcus aureus ATCC 12598	0.051(-)	1.069(+)
Vibrio cholerae ATCC 11623	0.052(-)	0.944(+)
Vibrio parahemolyticus ATCC 17802	0.053(-)	0.799(+)
Yersinia enterocolitica ATCC 23715	0.056(-)	1.016(+)

Cross Reactivity :

The **CULTURETTE**® BRAND *Toxin* **CD** Test reacts with all known toxigenic reference strains of *C. difficile.*[11] The test did not react with the nontoxigenic strain of VPI 11186. Cross reactivity was demonstrated to be negative with the bacteria shown in Table 6. Testing was performed by taking *C. difficile* toxin A negative and positive stools and adding the various organisms to final concentrations of 10^7 to 10^9 CFU/ml stool. As expected, the only organism shown to cross react in the test is a highly toxigenic isolate of *Clostridium sordellii* VPI 9048. This isolate elaborates high levels of hemorrhagic and lethal toxins which have been shown to be immunologically and biologically similar to *C. difficile* toxins A and B. The *Staphylococcus aureus* Cowan strain ATCC 12598, which produces protein A, did not show cross reactivity. Also, *Escherichia coil* ATCC 43889, 43894, and 43895 which produce Shiga-like toxins (SLT) did not show any cross reactivity.

XI. AVAILABILITY

Cat. No. Description

4954004 **CULTURETTE**® BRAND *Toxin* **CD**, 96 test kit
4364001 **CULTURETTE**® BRAND *Toxin* **CD**
4973888 **Becton Dickinson** Micropipettor, 100 ul fixed volume
4973310 Pipette Tips, box of 100

XII. REFERENCES

1. Sullivan, N.M., S. Pellett and T.D. Wilkins. 1982. Purification and characterization of toxin A and B of *Clostridium difficile.* Infect. Immun. **35**:1032-1040.

2. Lyerly, D.M., N.M. Sullivan and T.D. Wilkins. 1983. Enzyme-linked Immunosorbent assay for *Clostridium difficile* toxin A. J. Clin. Microbiol. **17**:72-78.
3. Bartlett, J.G. 1990. *Clostridium difficile:* clinical considerations. Rev. Infect. Dis. **12**:5243-S251.
4. Lyerly, D.M., H.C. Krivan and T.D. Wilkins. 1988. *Clostridium difficile:* its disease and toxins. Clin. Microbiol. **1**:1-18.
5. Baron, E.J. 1989. Assessment of currently-available laboratory tests for *Ciostridium* difficile-associated diarrhea. Clin. Microbiol. Newsl. **11**:118-120.
6. Cooperstock, M. 1988. *Clostridium difficile* in infants and children. *In* R.O. Rolfe, and S.M. Finegold (eds.). *Clostridium difficile:* its role in intestinal disease. Academic Press, Inc., San Diego, CA., p.45-64.
7. Peach, S.L., S.P. Borriello, H. Gayla, F.E. Barclay, and A.R. Welch. 1968. Asymptomatic carriage of *Clostridium difficile* in patients with cystic fibrosis. J. Clin. Pathol. **39**:1013-1018.
8. Lyerly, D.M., D.E. Lockwood, S.H. Richardson and T.D. Wilkins. 1982. Biological activities of toxins A and B of *Clostridium difficile.* Infect. Immun. **35**:1147-1150.
9. Gerding, D.M. 1989. Disease associated with *Clostridium difficile* infection. Ann. Intern. Med. **110**:255-257.
10. Delmee, M., B. Vandercam, V. Avesani and J.L. Michaux. 1987. Epidemiology and prevention of *Clostridium difficile* infections in a leukemia unit. Eur. J. Clin. Microbiol. **6**:623-627.
11. Data on file at Becton Dickinson Microbiology Systems.

TECHNICAL INFORMATION: In the United States telephone Becton Dickinson Microbiology Systems Technical Services, toll free (800) 638-8863. Prompt 2.

Approved By:_____

Supervisor:_____ Date:_____

Director:_____ Date:_____

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