

# COLOR PAC™ TOXIN A LABORATORY PROCEDURE

## I. INTENDED USE

**ColorPAC™** Toxin A Test is a rapid chromatographic assay for the qualitative detection of *Clostridium difficile* toxin A (enterotoxin) in stool specimens from patients suspected of having *C.difficile*-associated disease. The test can also be used for confirmation of suspect colonies of toxigenic *C.difficile* from agar plates or BHI broth. This assay is intended for use 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 with significant mortality. Although *C.difficile* may be 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.<sup>1</sup> The literature indicates that both toxin A and toxin B are produced at the same time.<sup>2</sup> 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 biological activity in the naturally-occurring disease.<sup>3</sup>

The most common clinical diagnostic aids for *C.difficile* antibiotic-associated colitis have been cell culture cytotoxicity assays (CTA), latex agglutination (LA), and enzyme immunoassays (EIA).<sup>4</sup> CTA detects toxin B through the cytopathic effect on cell culture and requires one to two 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 establishing whether there is an etiologic role for *C.difficile* in patients with diarrhea.<sup>5</sup> Microwell enzyme immunoassays can detect toxin A, or in some cases, both toxin A and toxin B simultaneously.<sup>6</sup>

## III. PRINCIPLE OF THE PROCEDURE

The **ColorPAC™** Toxin A Test is comprised of a capture *C.difficile* toxin A antibody immobilized on a chromatographic test strip, which is contained within a test device. A specimen (i.e. stool, colonies, or BHI broth) is diluted with sample buffer and added to the sample well of the test device. The diluted sample wicks up the test strip by capillary action. Toxin A, if present, binds to the capture antibody at the test line as the specimen migrates across the test strip. The wash and remaining reagents are added to the reagent well of the test device.

Upon addition of Detector A, toxin A antibody-coated liposomes containing a pink dye migrate across the test strip and attach to the *C.difficile* toxin A antigen that was bound to the test strip in the previous step. Color formation is enhanced upon addition of a second liposome reagent, Detector B that binds to the complex formed between Detector A and sample antigen at the test line. Following the final wash, the reactions are read visually. If toxin A antigen is detected in the specimen, a pink test line and a pink control line will appear, indicating a positive result. In the absence of toxin A, only the pink control line will appear, indicating a negative result.

#### IV. REAGENTS

##### ColorPAC™ Toxin A Test Kit:

<b>Reagent 1</b>	(30.0ml)	Sample Buffer: Buffered saline with a mucolytic agent, 1% detergent.
<b>Reagent 2</b>	(1.5ml)	Detector A: Rabbit <i>C.difficile</i> toxin A antibody-coated liposomes.
<b>Reagent 3</b>	(1.5ml)	Detector B: Rabbit antibody-coated liposomes specific for Detector A.
<b>Reagent W</b>	(2.3ml)	Wash Reagent: Buffered saline solution with bovine protein stabilizer, 0.1% detergent.
<b>Control +</b>	(1.0ml)	Positive Control: Buffered saline solution with bovine protein stabilizer, inactivated <i>C.difficile</i> toxin A.
<b>Control -</b>	(1.0ml)	Negative Control: Buffered saline solution with bovine protein stabilizer.

Reagents and Controls each contain 0.2% sodium azide (preservative).

30 Test Devices Each containing a test strip coated with monoclonal *C.difficile* toxin A antibody and *C.difficile* toxin A.

30 SQ-EASY™ Tubes and Filter Tips

30 each Applicator Sticks and Transfer Pipets

1 Dropper Rod

**Precautions:** For In Vitro Diagnostic Use.

**Reagents:** Upon receipt, refrigerate the kit or remove the carton of reagents requiring refrigeration and store at 2-8°C. DO NOT FREEZE. Reagents should be recapped immediately and returned to refrigeration when not in use, taking care not to mix color-coded caps. Do not use beyond the expiration date. Upon removal from the refrigerator, allow reagents to warm to room temperature before use. Avoid prolonged exposure of reagents to strong light.

Do not interchange, mix, or combine reagents and devices from different kit lots.

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

**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 Devices:** Do not remove test device from pouch until just prior to use. Store unopened devices at room temperature (15-30°C) or refrigerate (2-8°C) if desired. Do not expose unused devices to repeated cycling between refrigerated and room temperatures. Single use; do not reuse.

**Controls:** Do not use the kit if the positive and negative controls do not yield appropriate results. The positive control is made with inactivated *C.difficile* toxin A and should be handled as potentially hazardous material.

**Transfer Pipets:** Single use; do not reuse.

**Filter Tips:** Tip must contain white filter material to ensure proper test performance. Single use; do not reuse. Ensure that the filter tip snaps into place so that the top rim of the filter tip is flush with the top of the SQ-EASY tube.

Pathogenic microorganisms including Hepatitis B Virus and Human Immunodeficiency Virus may be present in specimens. “Universal Precautions” <sup>13,14</sup> and institutional guidelines should be followed in handling all items contaminated with blood or other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.
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Dispose of all materials used in performing the test into a receptacle approved for biohazardous waste. The samples may be autoclaved 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 minutes. *Do not autoclave materials containing sodium hypochlorite.*

## V. SPECIMEN COLLECTION AND HANDLING

Collect stool specimens into a clean airtight container with no preservative. Testing of specimens should be performed as soon as possible upon receipt in the laboratory; however, storage for up to 72 hours at 2-8°C is permissible. If specimens are to be tested after 72 hours, they should be frozen at -70°C immediately upon receipt in the laboratory. Specimens may be frozen up to 2 months. Positive specimens will show little or no reduction in toxin A detected by **ColorPAC** Toxin A after three freeze-thaw cycles. Allow stool specimens to warm to room temperature and mix as thoroughly as possible before use.

**ColorPAC Toxin A** test results are not affected by ampicillin, cephalixin, metronidazole, vancomycin, barium sulfate, laxatives, blood, and antidiarrheal medications that may be present in stool.

It is suggested that test for *C. difficile* or its toxins be performed on diarrheal (unformed) stool specimens.<sup>7</sup>

## **VI. PROCEDURE**

**Materials provided:** All materials as listed under “Reagents”.

**Materials Required But Not Provided:** Vortex mixer and timer, micropipettor; (optional: Brain Heart Infusion Broth or *C.difficile* selective agar needed only for toxin A detection in *C.difficile* culture isolates).

**Performance of Test:** The testing area, reagents, test specimens, and test components should be at room temperature prior to testing. Gently mix all reagents several times by inverting prior to use. Avoid foaming.

### **Stool Specimen Preparation**

1. With a micropipettor or supplied dropper rod, add 1ml of **Reagent 1** (Sample Buffer) to one SQ-EASY tube for each sample to be tested.
2. Mix stool specimen as thoroughly as possible. For liquid or semi-solid stools, pipet 0.5ml specimen into tube containing **Reagent 1** using a provided transfer pipet. For solid stools, use an applicator stick or wood spatula provided to pick up two pea size samples (0.5g) from separate areas of the specimen and transfer to the tube containing **Reagent 1**. Cap the tube with a filter tip prior to vortexing.
3. Vortex the tube for 15 sec at high speed. The diluted sample is ready for use. Proceed to “Assay Procedure”.

### **Colony and BHI Broth Specimen Preparation (i.e. optional)**

#### **Toxin A Testing from Plated Media Culture:**

1. Locate suspected colonies on the agar surface from 42-48 hours cultures meeting morphological and Gram stain characteristics of *C.difficile*.
2. With a micropipettor or supplied dropper rod, add 1ml of **Reagent 1** (Sample Buffer) to a 12x75 mm culture tube. Aseptically transfer suspected isolated colonies sufficient to achieve a 2.0 McFarland turbidity.

3. Make a visual comparison of the bacterial suspension to a 2.0 McFarland standard. Use additional **Reagent 1** to adjust to a 2.0 McFarland turbidity if necessary.
4. Vortex the tube for 15 sec at high speed. The diluted sample is ready for use.
5. Proceed to “Assay Procedure”.

#### **Toxin A Testing from BHI Broth:**

1. With a micropipettor or supplied dropper rod, add 1ml of **Reagent 1** (Sample Buffer) to a SQ-EASY tube.
2. Add 0.5ml of a 72 hours BHI broth with suspected *C.difficile* culture. Cap the tube with a filter tip prior to vortexing.
3. Vortex the tube for 15 sec at high speed. The diluted sample is ready for use.
4. Proceed to “Assay Procedure”.

#### **Assay Procedure:**

Open sufficient Test Devices for the number of samples and controls to be tested.

1. Add 3 drops of diluted sample (125µl) into the Sample Well of a Test Device. Allow sample to absorb for 3 minutes.

NOTE: On rare occasions, samples that do not pass through the filter tip or samples that filter but do not flow, will require centrifugation at a minimum of 1,500 x g for 10 min. Using a micropipettor, add 125µl of supernatant to the Sample Well of a new Test Device.

In place of the diluted sample, 2 drops of **Control+** or **Control-** can be added to the Sample Well for quality control.

2. Add 1 drop of **Reagent W** (Wash Reagent) into the Reagent Well and allow to absorb.
3. Add 1 drop of **Reagent 2** (Detector A) into the Reagent Well and wait 3 minutes.
4. Add 1 drop of **Reagent W** (Wash Reagent) into the Reagent Well and allow to absorb.

NOTE: For strongly reactive samples, a pink test line (positive) may appear in the Test Device window prior to completion of steps 5 and 6.

5. Add 1 drop of **Reagent 3** (Detector B) into the Reagent Well and wait 3 minutes.
6. Add 1 drop of **Reagent W** (Wash Reagent) into the Reagent Well and allow to absorb. Read the results after 1 minute in a well-lighted area. Color intensity and background may change over time, but results can be interpreted for an additional 10 minutes.

## VII. RESULTS

**Positive Test:** A result is **positive** if a pink test line **of any intensity** appears in the Assay Window (area “T”). A positive test result indicates that *C.difficile* toxin A has been detected in the patient specimen. A pink control line **of any intensity** should appear in the Assay Window (area “C”). Some intense positive reactions may cause a reduction in the control line intensity. The background intensity should not obscure the control line.

**Negative Test:** A result is **negative** if there is no visible test line in the Assay Window (area “T”). A negative test result indicates that *C.difficile* toxin A was not detected in the patient specimen. A pink control line **of any intensity** should appear in the Assay Window ( area “C”). Some patient specimens may cause a dark stained background, resulting in reduced control line intensity.

As with many diagnostic assays performed on stool for *C.difficile* toxin, if the result is negative, and if symptoms persist and *C.difficile*-associated diarrhea is suspected, testing of the same or subsequent stool specimens with a different methodology is recommended.

**Uninterpretable Test:** The result in **uninterpretable** if there is no pink control line in the Assay Window ( area “C”) or if the background obscures reading of the control line.

If the result is uninterpretable, a new sample should be prepared, centrifuged at a minimum 1,500 x g for 10 minutes, and tested as described in steps 1 through 6 of the “Assay Procedure”. If the sample remains uninterpretable, a new specimen should be requested.

## VIII. QUALITY CONTROL

The liquid **Control+** and **Control-** should be tested when opening each new kit to verify performance of the reagents and test device. Add 2 drops of the **Control+** or **Control-** to the sample well. Allow to absorb for 3 min. Proceed to step 2 under “Assay Procedure”.

**Optional:** Dilution of the **Control+** with **Reagent 1** may be performed to demonstrate a weaker positive reaction as follows:

1. Add 1.5ml of **Reagent 1** (Sample Buffer) and one free falling drop of **Control+** to a test tube. Mix gently.
2. Add 1.0 ml of **Reagent 1** (Sample Buffer) to an SQ-EASY tube.
3. Using a transfer pipet, transfer 0.5ml of diluted **Control+** from the first tube into the SQ-EASY tube.
4. Cap the SQ-EASY tube with a filter tip. Ensure that filter snaps into place.
5. Vortex the tube for 15 sec at high speed. The diluted sample is now ready for use. Proceed to “Assay Procedure”.

The **ColorPAC** Toxin A device contains two built-in controls. The appearance of a pink control line in the Assay Window (area “C”) provides an internal positive control that validates the immunological reactivity of the device, proper detection reagent function, and proper flow characteristics. The membrane area (background) functions as the internal negative control which assures that non-specific color development does not interfere with the test result.

Patient results should not be reported if positive and negative controls do not yield appropriate results.

Local, regional, or other laboratory regulations may apply which supersede package insert directions for frequency of testing the positive and/or negative controls.

## IX. LIMITATIONS OF THE PROCEDURE

The **ColorPAC** Toxin A Test does not define the presence of *C.difficile*-associated disease, but only demonstrates the presence of toxin A in the stool. The level of toxin has not been shown to be correlated with either the presence or absence of patient disease. These test results should be interpreted by a physician in conjunction with other laboratory test results and patient clinical findings.

The diagnosis of *C.difficile*-associated diarrhea (CDAD) should be suspected in any patient with diarrhea who has received antibiotics within the previous 2 months and/or whose diarrhea began 72hours or more after hospitalization.<sup>7</sup>

Factors such as technical or procedural errors, as well as additional substances present in the stool specimen that are not listed under “Specimen Collection and Handling”, may interfere with the test and cause erroneous results.

Some isolates of *Clostridium sordellii* have been shown to product 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.<sup>4</sup> The *C.sordellii* HT stains 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.<sup>8,9</sup>

Performance characteristics of the **ColorPAC** Toxin A test have not been established in a pediatric population (<12 yrs.).

Performance studies have not been conducted in a physician’s office laboratory or point of care setting.

As with other brands of tests, specimen handling is important for the maintenance of toxin A titers. If testing is delayed more than 72 h, freezing of samples at -70°C is recommended (see “Specimen Collection and Handling”).

## **X. 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.

Estimates indicate up to 15% of healthy adults may have positive stool cytotoxin tests.<sup>10,11</sup> The prevalence of *C.difficile* infection in diarrhea patients varies with the type of institution, housekeeping practices, and patient population. Prevalence rates ranging from 8.5% to 13.5% in patients suspected of *C.difficile*-associated disease were observed when the **ColorPAC** Toxin A test was evaluated with prospective specimens in clinical trials at four major independent medical centers.<sup>12</sup>

## XI. PERFORMANCE CHARACTERISTICS

The performance of the **ColorPAC** Toxin A test was determined in evaluations conducted at four major independent medical centers. The sites were located in geographically diverse areas of the United States. A total of 598 fresh and 162 frozen stool specimens from patients with suspected *C.difficile*-associated disease were tested. Each site compared its routine cytotoxin B assay to the **ColorPAC** Toxin A test. Only 0.1% of stools required centrifugation and repeat testing before obtaining the reportable result. Results of this initial comparison are summarized in Table 1.

**Table 1**  
**Comparison of ColorPAC Toxin A Results versus Cytotoxin B Results**

Site	No.	ColorPAC Toxin A	Cytotoxin B Results		95% Confidence Intervals
			Positive	Negative	
1	240	Positive	28	6	Sensitivity 82% (65.5%, 93.2%)
		Negative	6	200	Specificity 97% (93.8%, 98.9%)
2	135	Positive	18	1	Sensitivity 90% (68.3%, 98.8%)
		Negative	2	114	Specificity 99% (95.3%, 99.9%)
3	207	Positive	21	11	Sensitivity 81% (60.7%, 93.5%)
		Negative	5	170	Specificity 94% (89.4%, 96.9%)
4	178	Positive	29	1	Sensitivity 74% (57.9%, 87.0%)
		Negative	10	138	Specificity 99% (96.1%, 100%)
Combined	760	Positive	96	19	Sensitivity 81% (72.4%, 87.3%)
		Negative	23	622	Specificity 97% (95.4%, 98.2%)

Due to the published lack of standardization of cytotoxin assays, discordant specimens were further investigated with toxigenic culture. Comparison of **ColorPAC** Toxin A test discordant results with toxigenic culture is summarized in Table 2.

**Table 2**  
**Comparison of ColorPAC™ Toxin A Results versus Cytotoxin B**

**Results with Resolution of Nonconcordant Specimens**

Site	No.	ColorPAC Toxin A	Cytotoxin B Results	
			Positive	Negative
1	240	Positive	30	4
		Negative	5	201
2	135	Positive	18	1
		Negative	0	116
3	207	Positive	22	10
		Negative	0	175
4	178	Positive	29	1
		Negative	7	141
Combined	760	Positive	99	16
		Negative	12	633

As indicated in “Expected Values”, prevalence rates ranged from 8.5% to 13.5% in patients suspected of *C.difficile*-associated disease when the **ColorPAC** Toxin A test was evaluated with prospective specimens in clinical trials at four major independent medical centers. The positive and negative predictive values (PPV, NPV) for each site are shown in Table 3 based on actual prevalence and performance characteristics at each of the clinical sites.

**Table 3  
ColorPAC Toxin A – Positive and Negative Predictive Values  
Based on Actual Prevalence and Performance Characteristics**

Site	Sensitivity	Specificity	Prevalence	PPV	NPV
1	82%	97%	8.5%	72%	98%
2	90%	99%	13.5%	93%	98%
3	81%	94%	10.2%	61%	98%
4	74%	99%	11.1%	90%	97%

The **ColorPAC** Toxin A test was also compared to three Enzyme Immunoassays (EIA). Each of the four clinical trial sites tested at least one EIA, **ColorPAC** Toxin A and cytotoxin B for each sample. Results of each toxin A assay are compared to cytotoxin B and summarized in Table 4.

**Table 4  
Combined Sites Comparison of Toxin A Assays versus Cytotoxin B**

Performance	ColorPAC Toxin A	EIA Method 1	EIA Method 2	EIA Method 3
Sensitivity	81%	80%	74%	86%
Specificity	97%	97%	98%	98%
Agreement	95%	94%	94%	96%
% Initial Uninterpretable or Indeterminate	0.1%	0.9%	2.4%	0%

**XII. COLONY CONFIRMATION**

A study was conducted to evaluate the **ColorPAC** Toxin A test performance for providing culture confirmation from suspected colony growth for toxin producing

strains of *C.difficile* using selective agar, anaerobic blood agar, and Brain Heart Infusion (BHI) broth. A total of 111 clinical isolates that met morphological characteristics of *C.difficile* were tested from each media using the **ColorPAC** Toxin A test. After identification of the colonies by biochemical methods, toxigenic status was determined by a cytotoxin B assay. Results from the initial comparison are summarized in Table 5.

**Table 5**  
**Comparison of ColorPAC Toxin A Results versus Cytotoxin B – Colony Confirmation by Media Type (Initial Testing)**

Media Type	No. Specimens	Sensitivity		Specificity	
		95% Confidence Intervals		95% Confidence Intervals	
BHI Broth	111	52/55	95% (84.9%,98.8%)	52/56	93% (82.7%,98.0%)
Selective Agar	111	52/55	95% (84.9%,98.8%)	52/56	93% (82.7%,98.0%)
Anaerobic Blood Agar	111	49/55	89% (77.7%,95.9%)	53/56	95% (85.1%,98.9%)

Discordant results were further investigated by testing colonies for Toxin A production using a Toxin A EIA method. Comparison of **ColorPAC** Toxin A test discordant results to cytotoxin B and a Toxin A method are summarized in Table 6.

**Table 6**  
**Comparison of ColorPAC Toxin A Results versus Cytotoxin B – Colony Confirmation by Media Type (with Resolution)**

Media Type	No. Specimens	Sensitivity	Specificity
BHI Broth	111	55/55	55/56
Selective Agar	111	55/55	55/56
Anaerobic Blood Agar	111	52/55	56/56

**Cross Reactivity:** The **ColorPAC** Toxin A test was evaluated for cross reactivity by seeding microorganisms (i.e. bacteria, yeast, viruses, and parasites) into toxin A positive and toxin A negative stool specimens to a final concentration of  $10^7$ - $10^8$  CFU/ml for bacteria and yeast,  $10^{3.2}$ - $10^{6.2}$  TCID<sub>50</sub>/ml for viruses, and  $10^6$  parasites/ml. 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* toxin A and B. The *Staphylococcus aureus* Cowan strain ATCC 12598, which produces protein A, did not show cross reactivity. Also *Escherichia coli* ATCC 43889, 43894, and 43895 which product Shiga-like toxins (SLT) did not show any cross reactivity.

The following microorganisms did not give false positive results in toxin A negative stool or false negative results in toxin A positive stools.

Microorganisms (# Strains tested)	
<i>Adenovirus, types 2, 40, 41</i> (3)	<i>Cytomegalovirus</i> (1)

<i>Aeromonas hydrophilia</i> (1)	<i>Echovirus</i> , type 22 (1)
<i>Bacillus cereus</i> (1)	<i>Entamoeba histolytica</i> (1)
<i>Bacillus subtilis</i> (1)	<i>Enterococcus faecalis</i> ATCC 29212 (1)
<i>Bacteroides fragilis</i> (1)	<i>Enterococcus faecium</i> , ATCC 51559 (Vancomycin resistant) (1)
<i>Campylobacter coli</i> (1)	<i>Enterovirus</i> , type 69 (1)
<i>Campylobacter fetus</i> (1)	<i>Escherichia coli</i> (4)
<i>Campylobacter jejuni</i> (1)	<i>Giardia intestinalis</i> (1)
<i>Campylobacter laridis</i> (1)	<i>Klebsiella pneumoniae</i> (1)
<i>Candida albicans</i> (1)	<i>Peptostreptococcus anaerobius</i> (1)
<i>Clostridium botulinum</i> , type A (1)	<i>Proteus mirabilis</i> (1)
<i>Clostridium butyricum</i> (1)	<i>Pseudomonas aeruginosa</i> (1)
<i>Clostridium histolyticum</i> (1)	<i>Rotavirus</i> , human (1)
<i>Clostridium innocuum</i> (1)	<i>Salmonella choleraesuis</i> (1)
<i>Clostridium novyi</i> (1)	<i>Shigella dysenteriae</i> (1)
<i>Clostridium perfringens</i> (1)	<i>Shigella flexneri</i> (1)
<i>Clostridium sepicum</i> (1)	<i>Shigella sonnei</i> (1)
<i>Clostridium sordellii</i> , VPI 9048 (1)	<i>Staphylococcus aureus</i> , ATCC 12598 (Cowan) (1)
<i>Clostridium sporogenes</i> (1)	<i>Vibrio cholerae</i> (1)
<i>Clostridium subterminale</i> (1)	<i>Vibrio parahaemolyticus</i> (1)
<i>Clostridium tetani</i> (1)	<i>Yersinia enterocolitica</i> (1)
<i>Coxsackie virus B1</i> (1)	

### XIII. LIMIT OF DETECTION

The analytical sensitivity of **ColorPAC** Toxin A test was performed by seeding known concentrations of toxin A into five specimens each of liquid, semi-solid and solid stools. Seeded specimens were tested in triplicate. Results are summarized in Table 7.

**Table 7**  
**Limit Of Detection by Stool Types**

<b>Toxin A Seeded Matrix</b>	<b>LOD Range (ng/ml)</b>
Liquid Stool	1.38-5.21
Semi-Solid Stool	1.61-18.71
Solid Stool	3.19-22.58

### XIV. ASSAY REPRODUCIBILITY

Reproducibility of the **ColorPAC** Toxin A test was evaluated at several clinical laboratories by testing stool panels consisting of three levels of toxin A and a toxin A negative antigen control. Blinded specimens were tested in triplicate on each of three days at each of four different clinical trial sites. **ColorPAC** Toxin A demonstrated 100% intra- and inter-assay reproducibility.

## XV. AVAILABILITY

Catalog #	Description
4974030	<b>ColorPAC Toxin A, <i>C.difficile</i>, 30 Test Kit</b>

## XVI. REFERENCES

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**TECHNICAL INFORMATION:** In the United States, telephone Becton Dickinson Microbiology Systems Technical Services toll free (800) 638-8663, Prompt2.

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Date Effective: \_\_\_\_\_

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Director: \_\_\_\_\_ Date: \_\_\_\_\_

Reviewed:

PI Rev. 07/98  
Rev. 09/98