

## Revised Abstract

**Background:** A number of laboratory tests are available for diagnosing *C. difficile* infection (CDI). Toxin A/B EIA tests which have been used by most laboratories are no longer sensitive enough for primary testing. Recent recommendations include a combination of testing procedures that start with a sensitive screening method such as Glutamate Dehydrogenase EIA (GDH), also known as Common Antigen (CA), followed by confirmation of screen positive specimens using a test with higher specificity. GDH testing followed by Toxin A/B EIA of GDH(+) samples was used in our laboratory until recently. Approximately 50% of GDH(+) samples confirmed as Toxin A/B(+) by EIA, but half of the GDH(+)/Toxin A/B(-) samples contained A/B toxin when tested by more sensitive methods such as culture and PCR. Our previous evaluation of C. Diff Banana Broth™ (CDBB) (Hardy Diagnostics) showed it could resolve many of the GDH(+)/Toxin A/B(-) samples in our laboratory by stimulating toxin production during culture. The present study evaluates the new *illumigene*<sup>®</sup> *C. difficile* (ICD) molecular assay (Meridian Bioscience Inc.) by comparing its performance to CDBB, GDH, and Toxin A/B EIA testing. **Methods:** 126 fresh sequential patient specimens submitted over a 1 month period were tested for GDH followed by Toxin A/B EIA confirmation of GDH(+) specimens. In addition, all specimens were cultured in CDBB and tested using the ICD assay following manufacturer's procedures. CDBB tubes that turned yellow during incubation (mannitol utilization) were centrifuged at 3,000 rpm and the supernatant used to detect Toxins A/B by EIA. **Results:** Of 126 samples tested, 31 (24.6%) were GDH(+), 14 (11.1%) were Toxin A/B EIA (+), 22 (17.5%) were ICD(+), and 23 (18.3%) were CDBB(+) for toxigenic CD. Of 17 GDH(+)/Tox A/B(-) samples, 8 (47.1%) and 9 (52.9%) were positive by ICD and CDBB respectively. Only 3 of 95 GDH(-) specimens tested were positive by ICD or CDBB and chart review indicated no CDI symptoms among these patients. **Conclusions:** The sensitivity of the ICD assay was similar to CDBB culture. ICD appears to be a sensitive method compared to CDBB, and can be used as either a first line or confirmatory laboratory test for CDI detection.

## Introduction

*Clostridium difficile* infection (CDI) is the leading cause of nosocomial diarrhea in health care facilities. CDI infection rates have increased markedly over the past 10 years in the US and other countries primarily due to the emergence and spread of a new hypervirulent strain designated NAP1. Rapid and accurate laboratory diagnosis of CDI is critical for patient treatment and infection control measures. Current laboratory testing methods include EIAs for toxin A/B or for glutamate dehydrogenase (GDH) (also known as "common antigen"), cytotoxin neutralization, and newer Nucleic Acid Amplification Tests (NAAT). To date, no currently available laboratory test that detects either *Clostridium difficile* (CD) or its toxins from stool specimens is sufficiently sensitive and specific to be used for an accurate and low cost diagnosis of CDI. Toxin A/B EIA assays have been used predominantly but recent recommendations discourage their use due to the lack of sufficient sensitivity. Current recommendations include a combination of testing procedures that start with a screening method such as GDH and are followed by performance of a confirmatory test on stool specimens that screen positive. Unfortunately, many currently available confirmatory test methods either lack sufficient sensitivity (Toxin A/B EIAs) or are labor intense and may take several days to complete (Cytotoxin B assay or *C. difficile* culture). Newer NAAT procedures are more expensive to perform but can be used as either primary or confirmatory test procedures.

Until recently, the current protocol at our institution was to screen stool specimens using GDH EIA, followed by a Toxin A/B EIA assay to confirm GDH(+) samples. However, previous studies in our laboratory showed that 40-50% of GDH(+)/Toxin A/B(-) specimens still harbored toxigenic *C. difficile* that could only be detected by more sensitive but labor intense methods (i.e. cytotoxin, CD culture, or PCR). A previous evaluation of C. Diff Banana Broth™ (CDBB) (Hardy Diagnostics) showed that it could also resolve 40-50% of the GDH(+)/Toxin A/B(-) samples as being positive for CD toxins.

The new FDA approved *illumigene*<sup>®</sup> *C. difficile* assay (ICD) (Meridian Diagnostics Inc.) is based on Loop-Mediated Isothermal Amplification technology for the detection of a stable region of the toxin A gene (tcdA) in stool samples. Previous comparative studies have indicated sensitivities comparable to other FDA approved CD molecular assays.

The purpose of this study was to evaluate the ICD assay and to compare its performance to CDBB culture, GDH EIA, and Toxin A/B EIA testing.

## Materials and Methods

**Specimens:** 126 fresh, non-duplicate stool specimens were studied. All testing procedures were performed on the same day or within 48 hrs on refrigerated specimens. All specimens were tested by GDH EIA, ICD, and CDBB culture. Only GDH(+) specimens were further tested using Toxin A/B EIA.

**Enzyme Immunoassays (EIA):** Microtiter EIAs were performed for GDH and Toxins A/B using the C. DIFF CHEK™ -60 (Inverness Medical) and Premier™ Toxins A/B (Meridian Bioscience, Inc.) respectively following manufacturer's instructions.

**C. Diff Banana Broth™:** CDBB (Hardy Diagnostics) is a commercial media formulated to select, differentiate and presumptively identify *C. difficile* from stool samples. As growth of *C. difficile* occurs in the medium, a pH indicator turns visually yellow (Figure 1). CDBB is also formulated to stimulate toxin production by toxigenic *C. difficile* strains.

### Procedure

- Using sterile forceps, a supplement tablet was added to each tube of CDBB and allowed to dissolve for two minutes prior to specimen inoculation.
- Using a swab, tubes of CDBB were inoculated with a portion of patient stool by inserting the swab down to the bottom of the tube and rotating, taking care not to over agitate the broth.
- Tubes were incubated at 35°C
- Tubes that turned yellow (>50% of tube) were centrifuged for 5 minutes at 3,000 rpm.
- A portion of the supernatant was removed and used to perform *C. difficile* toxin A/B EIA testing in order to verify the presence of toxigenic *C. difficile* from the broth.

**illumigene<sup>®</sup>:** The ICD assay was performed according to the manufacturer's protocol. Magnesium-pyrophosphate produced during amplification results in turbidity which is read after 40 minutes incubation using the *illumipro*-10 Incubator/Reader supplied by the company (Figure 2).

## Results

126 patient stool samples were tested and 34 (27%) were positive for at least one test method (see Table 1). 31 of these 126 (24.6%) samples were positive for GDH, and further testing using Toxin A/B EIA showed that only 14 of 31 (45.2%) were also positive for Toxin A/B.

All 126 stool samples were tested using the ICD assay and the CDBB culture procedure. Of 31 GDH(+) samples, the ICD and CDBB methods were each positive for 21 (67.7%) samples. In other words, the ICD and CDBB methods increased the confirmed CD positive rate by 33.3%, over that of the Toxin A/B EIA.

Of 17 GDH(+)/Toxin A/B(-) samples, 8 (47.1%) and 9 (52.9%) were positive for ICD and CDBB respectively. There was only one GDH(+) specimen positive for Toxin A/B and CDBB but negative for ICD. Two GDH(+) specimens were positive for Toxin A/B and ICD, but negative for CDBB.

Only 3 of 95 GDH(-) specimens tested positive for ICD (1 sample) or CDBB (2 samples). Chart reviews did not reveal any evidence of CDI among these three patients prior to discharge.

	CDBB	ICD	Toxin A/B
Sensitivity	91%	91%	61%
Specificity	98%	99%	100%
PPV	91%	96%	100%
NPV	98%	98%	65%

## Acknowledgements

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## Table 1

Comparative Results of 34 Patient Samples Positive for at Least One Test Method Performed

Group	Patient No.	GDH	CDBB	ICD	Toxin A/B
A	A1	positive	positive	positive	positive
	A2	positive	positive	positive	positive
	A3	positive	positive	positive	positive
	A4	positive	positive	positive	positive
	A5	positive	positive	positive	positive
	A6	positive	positive	positive	positive
	A7	positive	positive	positive	positive
	A7	positive	positive	positive	positive
	A9	positive	positive	positive	positive
	A10	positive	positive	positive	positive
	A11	positive	positive	positive	positive
B	B1	positive	positive	negative	positive
C	C1	positive	positive	negative	negative
D	D1	positive	positive	positive	negative
	D2	positive	positive	positive	negative
	D3	positive	positive	positive	negative
	D4	positive	positive	positive	negative
	D5	positive	positive	positive	negative
	D6	positive	positive	positive	negative
	D7	positive	positive	positive	negative
	D8	positive	positive	positive	negative
E	E1	positive	negative	positive	positive
	E2	positive	negative	positive	positive
F	F1	positive	negative	negative	negative
	F2	positive	negative	negative	negative
	F3	positive	negative	negative	negative
	F4	positive	negative	negative	negative
	F5	positive	negative	negative	negative
	F6	positive	negative	negative	negative
	F7	positive	negative	negative	negative
	F8	positive	negative	negative	negative
G	G1	negative	positive	negative	ND
	G2	negative	positive	negative	ND
H	H1	negative	negative	positive	ND

## References

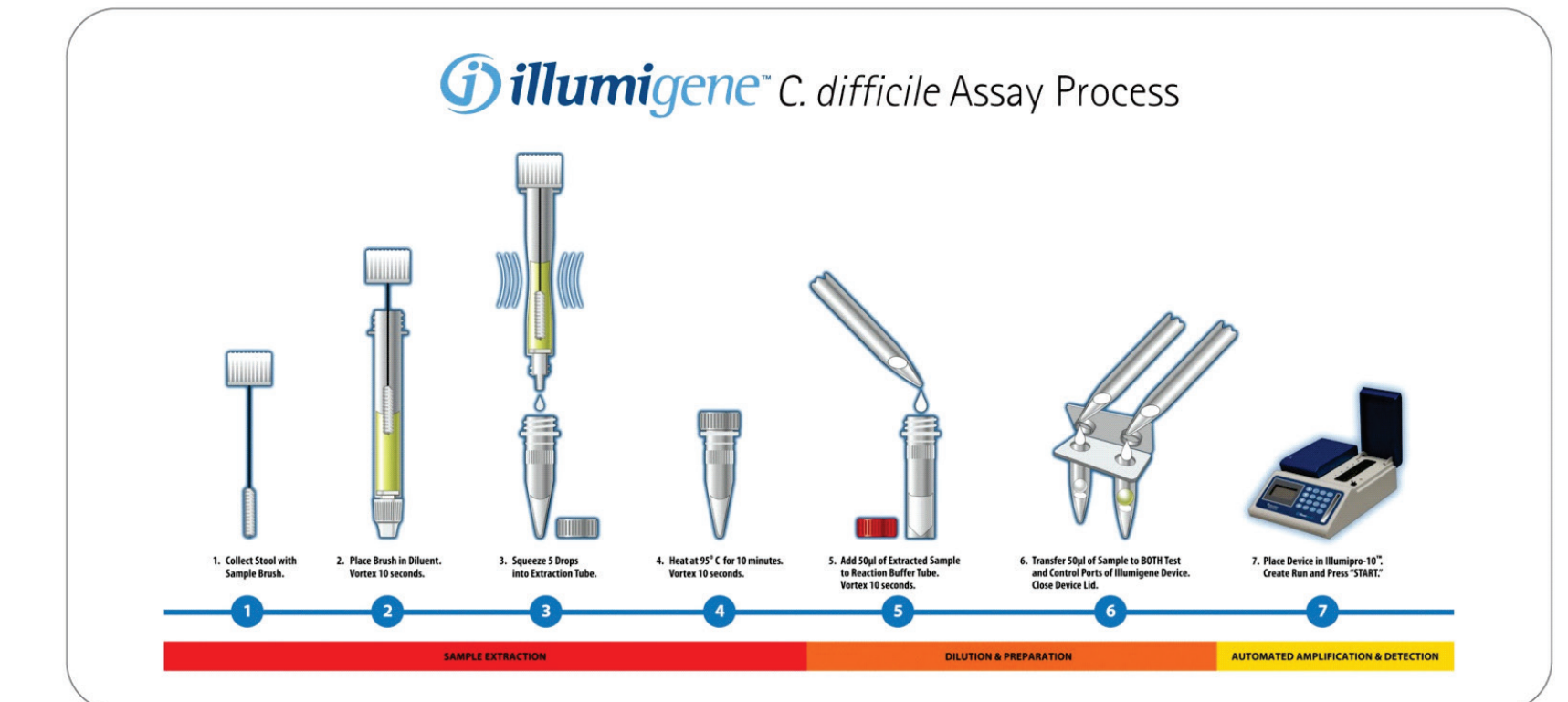
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## Figure 1



Positive and Negative CDBB Tubes

## Figure 2



## Discussion/Conclusions

- Of 31 GDH(+) stool samples, Toxin A/B EIA, ICD, and CDBB were positive in 14 (45.2%), 21 (67.7%), and 21 cases, respectively. ICD and CDBB increased the confirmed positive rate by 33.3% over Toxin A/B testing.
- Of 17 GDH(+)/Toxin A/B(-) samples, ICD and CDBB were positive in 8 (47.1%) and 9 (52.9%) cases, respectively.
- Only 3 of 95 GDH(-) patient specimens were positive by ICD (1 specimen) or CDBB culture (2 specimens) and chart review failed to detect any evidence of CDI prior to discharge. GDH EIA thus appears to be a sensitive and cost effective screening method in this study sample.
- The performance characteristics of ICD and CDBB are comparable. The ICD assay was easy to perform on both small and larger batches with results in 1 to 1½ hours.