immunocard STAT!®

C. difficile GDH-AB

Rapid one-step Immunoassay for the Simultaneous Detection of *Clostridium difficile* Glutamate Dehydrogenase Antigen (GDH) and Toxins A and B in human Stool

REF **750520** IVD

INTENDED USE

Immunocard STAT! *C. difficile* GDH-AB is a rapid, qualitative, immunochromatographic assay for the simultaneous detection, in human stool, of *Clostridium difficile* Glutamate Dehydrogenase antigen (GDH, also called "common antigen") and *Clostridium difficile* Toxins A and B. This assay is used as an aid in the diagnosis of *C. difficile*-associated disease and results should be used by the clinician in conjunction with clinical picture, other laboratory findings and epidemiological risk factors.

SUMMARY AND EXPLANATION OF THE TEST

Clostridium difficile is a spore-forming, gram-positive, anaerobic bacterium that can be present asymptomatically in up to 5% of the healthy population[1]. Toxigenic strains of *Clostridium difficile* represent the leading cause of nosocomial infectious diarrhea in developed countries, also representing the etiologic agent in approximately 25% of all cases of antibiotic-associated diarrhea[2]. An estimated 300,000 cases of *C. difficile* infections (CDAD) are seen per year in U.S. hospitals alone.[1, 2] Virtually any antibiotic can predispose a patient to CDAD. The clinical picture for CDAD ranges from asymptomatic colonization to life-threatening pseudomembranous colitis and toxic megacolon.[2] The pathogenic strains of *C. difficile* produce at least one of two biologically and immunologically distinct toxins[3]: toxin A (enterotoxin) and toxin B (cytotoxin), which are the main virulence factors responsible for the clinical signs of the disease.

Glutamate dehydrogenase (GDH), is an enzyme produced in large quantities by toxigenic and non-toxigenic strains of *C. difficile*, thus making it an excellent marker for determining the presence of this microorganism itself[4].

The Society for Healthcare Epidemiology of America (SHEA), the Infectious Diseases Society of America (IDSA) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) recommend different testing approaches for the laboratory detection of toxigenic *C. difficile*[5], including different combinations of GDH, toxins and NAAT assays. One of the suggested testing algorithms consists in the use of a two-step protocol, involving an initial screening of the sample with a GDH test and, if the result is positive, use of a second test to confirm the toxinogenity of the strain, as only the toxigenic and pathologic strains of this microorganism produce them. Immunocard STAT! *C. difficile* GDH-AB enables a lab to adopt this approach, obtaining the two results simultaneously with a single-step assay.

A positive result in the test for the glutamate dehydrogenase of *C. difficile* confirms the presence of this organism in a fecal specimen; a negative result indicates the absence of the organism. A positive result in the test for toxins A and/or B confirms the presence of toxigenic *C. difficile.*

BIOLOGICAL PRINCIPLES

The test Immunocard STAT! *C. difficile* GDH-AB contains two strips placed in a double cassette, one for GDH and one for Toxins A & B:

1. GDH strip uses a combination of:
    a. Pink-Red latex particles conjugated to specific antibodies against *C. difficile* GDH and GDH specific antibodies immobilized in the Test Position, below the Control Band. During incubation, if GDH antigen is present, is bound to both the antibodies, developing a pink-red line at Test Position.
    b. Blue latex particles conjugated to an antigen recognized by a specific antibody for this antigen, bound to the membrane, defining the Control Band of the test.
2. TOXINS strip uses a combination of:
    a. Pink-Red latex particles conjugated to specific antibodies against toxin B and antibodies specific for toxin B immobilized in B Position, below the Control Band. During incubation, if Toxin B is present, is bound to both the antibodies, developing a pink-red line at B Position.
    b. Pink-Red latex particles conjugated to specific antibodies against toxin A and antibodies specific for toxin A immobilized in A position, below the toxin B band. During incubation, if Toxin A is present, is bound to both the antibodies, developing a pink-red line at A Position.
    c. Blue latex particles conjugated to an antigen recognized by a specific antibody for this antigen, bound to the membrane, defining the Control band of the test.

    A diluted patient stool sample is dispensed into both sample ports of the cassette and migrates along the membranes through the Test and Control zones. After 15 minutes of incubation at room temperature, the appearance of a specific colored line in the reading window next to the corresponding letter indicates a positive result in the presence of the Control line (see Fig. 1).

REAGENTS/MATERIALS PROVIDED

The maximum number of tests obtained from this kit is listed on the outer box.

1. Immunocard STAT! *C. difficile* GDH-AB device: Two reactive strips housed in a plastic frame and enclosed in a foil pouch with a desiccant.
2. Sample Diluent vials: Buffered solution containing 0.095% of sodium azide as preservative. The diluent is supplied in a single use plastic dropper vial with an applicator stick. Use as supplied.
3. Disposable Transfer pipettes

MATERIALS NOT PROVIDED

1. External Control Set with Positive and Negative Controls. Meridian Cat. No 750501
2. Vortex
3. Interval Timer

PRECAUTIONS

1. All reagents are for in vitro diagnostic use only.
2. Do not deviate from the method described here or falsely positive or falsely negative results may occur. Once the assay has started, complete all subsequent steps without interruption.

3. Patient specimen and used Immunocard STAT! *C. difficile* GDH-AB device may contain infectious agents and should be handled at Biosafety Level 2 as recommended in the CDC/NIH manual "Biosafety in Microbiology and Biomedical Laboratories".
4. Do not interchange reagents from different kit lot numbers and do not use expired reagents.
5. Do not use the sample dilution buffer if there is evidence of contamination or precipitation.
6. The sample dilution buffer contains sodium azide which is a skin irritant. Avoid skin contact with reagents. Disposal of reagents containing sodium azide into lead or copper plumbing can result in the formation of explosive metal azides. This can be avoided by flushing with a large volume of water during such disposal.
7. Correct stool storage and correct stool dilution are essential to ensure correct results. Over-inoculation of stool into the Sample Diluent may restrict the flow within the Immunocard STAT! *C.difficile* GDH-AB device, determining invalid results. Incorrect stool storage or under-inoculation into the Sample Diluent may determine false negative results.
8. In case the primary packaging is damaged (foil pouch or diluent buffer vial) the product should be discarded and not used.
9. Do not use this product if a colored line appears in the result area of any strip before you start to use it.
10. If the test is stored refrigerated, allow all the kit components and faecal samples reach room temperature, because cold reagents and/or samples can reduce test functionality.
11. Do not discard the kit box until all the content has been used. This box contains essential information about the CE mark of the product and the batch number.
12. The used product should be discarded in compliance with current local laws

HAZARD AND PRECAUTIONARY STATEMENTS
There are no known hazards associated with this product.

SHELF LIFE AND STORAGE
Store the Immunocard STAT! *C. difficile* GDH-AB kit at 2-30 C when not in use. The kit expiration date is indicated on the kit label.

PROCEDURAL NOTES
1. Allow kit components and specimens to reach room temperature (19-27 C) before performing a test, as cold reagents and/or specimens may decrease assay sensitivity. Reagents may take up to 60 minutes to warm up following refrigeration.
2. Stool samples must be mixed thoroughly (regardless of consistency) prior to sampling to ensure a representative sample.
3. Hold reagent vials vertically when dispensing drops to ensure consistent drop size and delivery.
4. On occasion, particulate matter may interfere with sample flow. In cases when the Test Device does not readily absorb the diluted specimen, gently touch the bottom of the sample port with an applicator stick, moving the stool solid particle that might prevent the absorption. Alternatively, a new aliquot of the sample can be withdrawn from the Diluent and retested. Diluted samples containing a heavy concentration of particulate matter may be centrifuged (1-5 minutes at 700 x G) or allowed to stand for 3-5 minutes before proceeding.
5. Ensure to take the appropriate amount of sample: about 110 mg for solid samples (a small portion of about 5 mm diameter). If the sample is semi-liquid (unable to take it with a pipette) take an amount adequate of covering the grooves of the stick attached to the vial cap. If the sample is liquid, collect 110 µL (4 drops if the disposable pipettes provided with the kit are used). These amounts are extracted into the sample dilution buffer supplied in the vials included in the kit. An excess of sample in relation to the previously indicated may prevent the chromatography from running correctly; this is especially critical with solid samples since it is not easy to take the recommended amount of sample.
6. Ensure to add the correct volume of the extracted sample to the two sample ports marked with an arrow in the plastic device. If the volume is lower than indicated, the flow may not occur due to insufficient sample reaching the reaction strips; if the volume is higher, brown lines may appear instead of the expected ones (see Fig. 1).

REAGENT PREPARATION
Reagents are supplied ready to use. Allow kit components and specimens to reach room temperature (19-27 C) prior to use. Gently mix liquid reagent prior to use. Open the device pouch only when ready to run the assay.

SPECIMEN COLLECTION AND STORAGE
The test is validated for fresh untreated samples. Do not use samples collected in transport media, or those with added preservative agents (such as formalin, SAF, PVA or similar) or enrichment media, as their presence could interfere with correct performance of the test.

Stool samples should be transported in an airtight container. The sample should be tested as soon as possible but may be held up to 2 days at 2-8 C prior to testing. If testing cannot be performed within this timeframe, samples should be frozen immediately upon receipt and stored frozen (-20 C) until tested. Samples may be frozen and thawed twice. Ensure the samples have reached room temperature before testing.

TEST PROCEDURE
Bring all test cards, sample diluents and samples to room temperature (19-27 C) before testing. Remove the reagents from the kit box to warm. Reagents may take up to 60 minutes to warm following refrigeration.
1. Label one Sample Diluent Vial for each patient sample to be tested.
2. Unscrew the cap from the vial with caution in order not to spill the sample diluent buffer.
3. Immediately add stool sample or controls as follows:
   a. Formed/Solid stools – Mix the stool sample thoroughly. Using the stick attached to the vial cap, collect a small portion of 5 mm diameter.
   b. Semi-solid stools - Mix the stool sample thoroughly. Using the stick attached to the vial cap, collect a sample amount that completely covers the grooves of the stick.
   c. Liquid Stools - Using the disposable pipettes provided in the kit, mix the stool sample thoroughly, collect and dispense in the vial 4 drops of stool (corresponding to a volume of a 110 µL).
   d. External Positive or Negative Control: refer to EXTERNAL CONTROL SET Instructions for Use.
4. Carefully add the sample into the corresponding vial containing the dilution buffer. Screw the cap firmly and shake it vigorously to ensure mixture homogenization.
5. Use 1 Immunocard STAT! Test Card for each Sample or Control. When ready to perform testing, remove the Test Card from its foil pouch. Discard the pouch and desiccant. Label the device with the name of the patient or the control.
6. Break the top of the vial cap using a piece of paper to prevent leakage.
7. Invert the vial and, keeping it in a vertical position, add 4 drops of diluted sample in the Sample Port of each strip (rectangular windows marked with an arrow).
8. Incubate the test card at 19-27 C for 15 minutes.
9. Visually read the results of each card within 30 seconds at the end of incubation.

INTERPRETATION OF RESULTS

The cassette contains two strips: on the left side the strip for GDH, on the right side the strip for toxins A and B.
The possible outcomes are shown in Fig. 1.

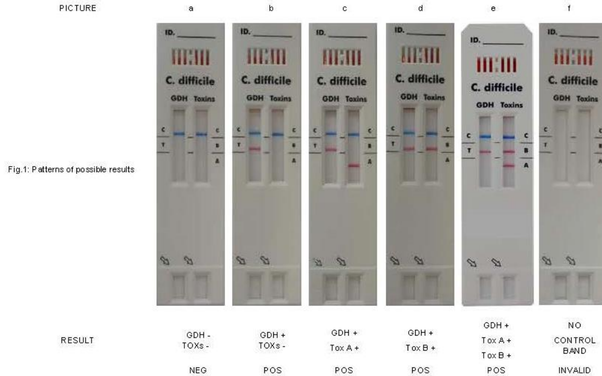

Fig.1: Patterns of possible results

NEGATIVE RESULTS: A **BLUE** band at the Control Line position (C). No other bands are present in the two strips of the cassette.
A negative result in the GDH antigen strip indicates *C. difficile* antigen either is absent in the specimen or is below the detection limit of the assay.
A negative result in the Toxins strip indicates either *C. difficile* toxins are absent in the specimen or they are below the detection limit of the assay.

POSITIVE RESULTS FOR GDH: A **PINK-RED** band at the TEST (T) Line Position, in the presence of a **BLUE** band at the Control Line Position (C) in the LEFT STRIP of the Cassette. A positive result indicates the presence of *C. difficile* in the sample.

POSITIVE RESULTS FOR TOXINS: A **PINK-RED** band at the TOXIN A (A) and/or the TOXIN B (B) Line Position, in the presence of a **BLUE** band at the Control Line Position (C) in the RIGHT STRIP of the Cassette. A positive result indicates the presence of *C.difficile* toxin(s).

INVALID RESULTS:

- For each strip, No **BLUE** band at the designated position for the Control Line (C). The test is invalid since the absence of a control band indicates that the test procedure was performed improperly or deterioration of reagents has occurred. For reporting a valid result, the **BLUE** bands in both strips should always appear.
- A **PINK-RED** band appearing at either the GDH, toxin A or toxin B test line position of the device **after** the defined incubation limit of 15 minutes or a band of **any color other than PINK-RED**. Falsely positive results may occur if tests are incubated too long. Bands with colors other than pink-red may indicate reagent deterioration or interference.

If any result is difficult to interpret, the test can be repeated with the same sample. Obtain a new sample and retest when the original sample repeatedly produces unreadable results.

Low percentage of specimens may test negative for antigen but positive for toxin(s). These samples should be retested using a fresh specimen. If new sample results negative for antigen but positive for toxin, report as positive toxin(s) result.

QUALITY CONTROL

This test should be performed per applicable local, state, or federal regulations or accrediting agencies.
At the time of each use, kit components should be visually examined for obvious signs of microbial contamination, freezing, leakage or any damage.

INTERNAL CONTROLS: Internal controls are contained within the test strip and therefore are evaluated with each test.

- The **BLUE** bands appearing at the Control Line Positions serve as a procedural control and indicate that the test has been performed correctly, proper flow occurred, and the test reagents were active at the time of use.
- A clean background around the Control and Test Lines also serves as a procedural control. Control or test lines that are obscured by heavy background color may invalidate the test and may be an indication of reagent deterioration, use of an inappropriate sample or improper test performance.

EXTERNAL CONTROLS: External Positive and Negative Controls should be assayed with each new kit lot or new shipment. The number of tests performed with the external controls will be determined by the requirements of local, state, federal regulations or accrediting agencies. External controls are used to monitor reagent reactivity and test performance. Failure of the controls to produce the expected results can mean that one of the reagents or components is no longer reactive at the time of use, the test was not performed correctly or that reagents or samples were not added.

The results expected with the controls are described in the section INTERPRETATION OF RESULTS. If the expected control reactions are not observed, repeat the control tests as the first step in determining the root cause of the failure. The kit should not be used if control tests do not produce the correct results.

EXPECTED VALUES

The frequency of antibiotic-associated diarrhea caused by *C. difficile* is dependent on several factors, including patient population, type of institution and epidemiology. The reported incidence of *C. difficile*-associated disease in patients suspected of having antibiotic-associated diseases is 15-20%, although different facilities may find positive rates above or below this range.

LIMITATIONS OF THE PROCEDURE

1. The assay is intended to confirm the presence of the GDH common antigen and toxins in patient's stool. A Negative Test result does not completely preclude an infection of a toxigenic *C. difficile* strain. Assay results are to be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures.
2. The test is qualitative and no quantitative interpretation should be made with respect to the intensity of the positive line when reporting the result.
3. The test is intended to be used on unpreserved human stool; any other kind of sample has not been validated.

4. Correct stool preservation is essential for reliable results. Sample degradation may determine False Negative results. While relatively stable at 2-8 C, *C. difficile* toxins - particularly at low concentrations - easily degrade at room temperatures. The rate at which the toxins degrade differs from patient sample to patient sample and therefore the rate cannot be predicted. For this reason, best practice requires that samples be refrigerated or frozen within two hours of collection and the samples tested within the timeframes recommended in this insert. Do not accept samples that have not been collected, handled or transported properly.
5. Two distinct groups have been identified that can harbor *C. difficile* asymptomatically at very high rates. Colonization rates of up to 50% and higher have been reported in infants and rates of up to 32% in cystic fibrosis patients.
6. Failure to add the correct amount of stool to the Sample Diluent Vial can lead to falsely negative or falsely positive results.
7. Over incubation of tests may lead to false-positive test results. Incubating tests at reduced temperatures or times may lead to falsely negative results.
8. Highly hemorrhagic samples may interfere with the assay by determining a false negative result or the appearance of aspecific bands. This event is often accompanied by the alteration of the color of the bands. Do not report any result if the bands are not of the correct color (Control Lines must be **BLUE,** Test Lines must be **PINK-RED**) – Refer to Fig. 1.
9. Cross reactivity studies indicated that stool samples strongly positive for *E. histolytica* might interfere with the results, determining a weak positive result for Toxin B.

SPECIFIC PERFORMANCE CHARACTERISTICS
Clinical performances of Immunocard STAT! *C. difficile* GDH-AB have been evaluated on a total of 142 samples for GDH and 138 samples for Toxins A and B. Specimen have been collected retrospectively and stored frozen. The samples have been characterized using commercial ELISA assay for GDH and for TOXINS A&B. Comparative results for Immunocard STAT! *C. difficile* GDH-AB are reported below:

	Immunocard STAT! C.difficile GDH-AB GDH STRIP		
REFERENCE METHOD: ELISA	**POS**	**NEG**	**TOTAL**
POSITIVE	38	2	40
NEGATIVE	1	101	102
Total	39	103	142
		%	CI 95%
Clinical sensitivity- Positive Agreement	38/40	**95,0**	83,5-98,6
Clinical specificity – Negative Agreement	101/102	**99,0**	94,6-99,8
Positive Predictive Value (PPV)	38/39	**97,4**	86,8-99,5
Negative Predictive Value (NPV)	101/103	**98,1**	93,1-99,5
	Immunocard STAT! C.difficile GDH-AB TOXINS A&B		
REFERENCE METHOD: ELISA	**POS**	**NEG**	**TOTAL**
POSITIVE*	35	2	37
NEGATIVE	0	101	101
Total	35	103	138
		%	CI 95%
Clinical sensitivity – Positive Agreement	36/38	**94,6**	82,3-98,5
Clinical specificity – Negative Agreement	101/101	**100**	94,3-100
Positive Predictive Value (PPV)	35/35	**100**	90-100
Negative Predictive Value (NPV)	101/103	**98,1**	93,2-99,5

*POSITIVE RESULTS confirmed by CCTNA (cell cytotoxicity & neutralization assay)

ANALYTICAL SENSITIVITY
Limit of Detection of the assay (LoD) has been determined as follows:

- GDH assay: two different preparations of glutamate dehydrogenase (native and recombinant) from *C. difficile* were used to determine the LoD value. A mean value of 0.8 ng/mL was obtained with both preparations.
- TOX A&B assay: toxins A and B from different sources (List Biological Laboratories, tgc BIOMICs and The Native Antigen) were used to determine the LoD value. A mean value of 12.5 ng/mL for toxin A and a mean value of 1.5 ng/mL for toxin B were obtained.

The lower LoD of the test was also determined using real stool samples as matrix. Values were consistent with those obtained as described above.

REPRODUCIBILITY
The reproducibility of the Immunocard STAT! *C. difficile* GDH-AB was determined using a single lot of the assay.

INTER-DAY PRECISION
The inter-day precision was measured by preparing serial dilutions (sensitivity curves) for each analyte. The same operator performed testing on four different days with a concordance of 100% in results.

INTER-OPERATOR PRECISION
Inter-operator precision was determined using serial dilutions for each analyte tested in duplicate by five operators on the same day. Differences were observed but in no case exceeded 1 two-fold dilution. The differences were considered as acceptable for a qualitative immunochromatographic technique.

Using a single lot of the assay, a sensitivity curve was measured for each analyte through four days spaced in time. The same sensitivity for GDH and both toxins A and B was obtained.

INTER-OPERATOR PRECISION:
Five operators measured in duplicate a sensitivity curve for each analyte. Differences were observed but in no case exceeded 1 two-fold dilution.

INTER-BATCH PRECISION:
Three different batches of the Immunocard STAT! *C. difficile* GDH-AB test were used to measure a sensitivity curve in duplicate. A single person performed the analysis on the same day. Differences of a dilution factor of 2 were observed, which are acceptable and tolerable for this test.

The differences found in the "Reproducibility" sections are acceptable for a qualitative immunochromatographic technique with its inherent variability.

PROZONE / HOOK EFFECT
Very high concentrations of the three analytes detected by the assay have been tested without observing any decrease in the intensity of the positive signals. These concentration values (higher than the maximum values that can be found among the population) are the following ones:
- GDH: 4000 ng/mL, about 1000-fold the assay LoD.
- Toxin A: 5000 ng/mL, about 400-fold the assay LoD.
- Toxin B: 5000 ng/mL, about 1500-fold the assay LoD

INTERFERING SUBSTANCES
The substances indicated in the below table at the concentration specified did not interfere with the results of the test when added to stool samples (positive and negative ones):

Racecadotril	5% {w/v}	Ibuprofen	20% {w/v}
Cimetidine	10% {w/v}	Acetylsalicylic Acid	30% {w/v}
Loperamide	5% {w/v}	Artificial sweetener	5% {w/v}
Metronidazole	10% {w/v}	Palmitic Acid	40% {w/v}
Omeprazole	3% {w/v}	Barium Sulfate	5% {w/v}
Ampicillin	15% {w/v}	Mucin	5% {w/v}

CROSS REACTIVITY
Cross reactivity of the assay was evaluated for different bacteria that can be present in the intestinal tract.

The assay was used to analyze real stool samples that were known to be strongly positive for the following bacteria/viruses and parasites: Adenovirus - Rotavirus - Norovirus - Astrovirus - *Helicobacter pylori - Entamoeba histolytica – Giardia lamblia - Cryptosporidium parvum*

A cross-reaction with stool containing *Entamoeba histolytica* has been registered. When the test is used on the isolated parasite (without the stool matrix) no cross reaction is detected. *Refer to Limitation of the Procedure, Point 9.*

Additionally, Stool samples inoculated with the following microbial agents (to a final sample concentration of ~ 1 x 10^8 organisms/mL) do not react with the assay:

Aeromonas caviae, Aeromonas hydrophila, Bacillus cereus, Bacillus spp, Bacteroides nordii, Campylobacter jejuni, Candida albicans, Clostridium butyricum, Clostridium cadaveris, Clostridium perfringens, Clostridium sordellii, Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli 1, Escherichia coli 2, Klebsiella pneumoniae, Lactobacillus gosseri, Listeria monocitogenes, Plesiomonas shigelloides, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella enteriditis, Salmonella typhi, Salmonella typhimurium, Serratia marcescens, Shigella dysenteriae, Shigella flexnerii, Shigella sonneii, Staphylococcus aureus, Staphylococcus epidermidis, Vibrio cholerae, Vibrio parahaemolyticus, Yersinia enterocolitica.

Additional information regarding the performance characteristics of this product can be obtained by contacting Meridian's Technical Support Department at the addresses reported below.

ITALIANO

immunocard STAT!®
C. difficile GDH-AB

Test immunologico rapido per il rilevamento simultaneo dell'antigene GDH (glutammato deidrogenasi) e delle tossine A e B di *Clostridium difficile* nelle feci umane

REF **750520** IVD

FINALITÀ D'USO
Immunocard STAT! *C. difficile* GDH-AB è un test rapido, qualitativo immunocromatografico per il rilevamento simultaneo, nelle feci umane, dell'antigene GDH (glutammato deidrogenasi, noto anche come "antigene comune") di *Clostridium difficile* e delle tossine A e B di *Clostridium difficile.* Questo test è da utilizzarsi come ausilio nella diagnosi di malattia associata a *C. difficile*; il medico dovrà valutare i risultati unitamente al quadro clinico, ad altri risultati di laboratorio e ai fattori di rischio epidemiologico.