EPITOPE DIAGNOSTICS, INC.

EDI™ Fecal ClostridiumDifficile GDH Antigen CLIA Kit

Chemiluminescence Immunoassay (CLIA) for the Quantitative Measurement of Clostridium Difficile GDH Antigen in Feces.

REF CL0824 CE IVD 100, 150, 250 +2 +8 °C

INTENDED USE

This Chemiluminescence Immunoassay (CLIA) kit is intended for the quantitative determination of Clostridium Difficile GDH (*C. Difficile*) antigen levels in feces using the ECL100 or ECL25 Immunoassay analyzer. It is for in-vitro diagnostics use only.

SUMMARY OF PHYSIOLOGY

Clostridium Difficile is a gram-positive anaerobe. Infection with C. Difficile causes severe diarrhea and can be fatal if not diagnosed and treated in a timely manner. C. Difficile infection is induced in patients by long-term treatment with antibiotics and is commonly found in hospital environment. It is easily transmitted through contact with infected fecal matter.

Since all strains of C. Difficile produce large amounts of glutamate dehydrogenase, testing for this antigen has proven to be a better screening tool due to its higher negative predictive value.

ASSAY PRINCIPLE

This CLIA is designed, developed, and produced for the quantitative measurement of *GDH* antigeninfecal samples. The assay utilizes a two-site “sandwich” technique with two antibodies that bind to different epitopes of *GDH antigen*.

Assay calibrators, controls, or extracted patient fecal samples are added directly to a reaction vessel. Simultaneously, a biotinylated antibody, a streptavidin coated magnetic particle and subsequentlyanacridinium ester conjugated antibodyare added to the vessel. The magnetic particles capture the biotin antibody as well as an immuno complex in the form of “magnetic particles – biotinylatedanti-GDH antibody –GDH antigen– acridinium ester conjugated anti-GDH antigen antibody”.

The materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the trigger solution is added to the reaction vessel and light generated by the reaction is measured with the ECL100 or ECL25 analyzer. The relative light units (RLU) are proportional to the concentration of GDH antigen in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in fecal C. Difficile antigen concentration.

REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.Reagents from different kit lot numbers should not be combined or interchanged.

1. GDH Magnetic Particle Solution (L0548)

Qty: 1 x 2.8 mL (100/kit), 2 x 2.5 mL (150/kit), 3 x 2.5 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

2. Biotin GDH Antibody (L0549)

Qty: 1 x 11 mL (100/kit), 1 x 16.5 mL (150/kit), 1 x 26.5 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

3. Acridinium Ester GDH Antibody (L0550)

Qty: 1 x 11 mL (100/kit), 1 x 16.5 mL (150/kit), 1 x 26.5 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

4. Fecal GDH Antigen Calibrators (L0559 – L0560)

Liquid Fecal GDH in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.
Qty: 2 x vials 0.5 mL each
Storage: 2 – 8°C
Preparation: Mix by inversions or gentle vortexing.

5. Fecal GDH Antigen Controls (L0561 – L0562)

Liquid fecal GDH in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.
Qty: 2 x vials of 0.5 mL each
Storage: 2 – 8°C
Preparation: Mix by inversions or gentle vortexing

6. Concentrated Fecal Extraction Buffer (30669) (Packaged Separately)

Concentrated buffer matrix with protein stabilizers and preservative which serves as a patient sample diluent containing a surfactant in phosphate-buffered saline with a non-azide preservative.
Qty: 1 x 60 mL
Storage: 2 – 8°C
Preparation: 4X Concentrate. The contents must be diluted with 180 mL distilled water and mixed well before use.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for in vitro diagnostic use. Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

The instrument only uses materials supplied by Epitope Diagnostics, Inc. When materials available from third-party suppliers are used, Epitope Diagnostics, Inc. takes no responsibility for the validity of results obtained. Material is available for purchase from Epitope Diagnostics, Inc. Please contact your distributor for more information.

1. Immunoassay Analyzer (ECL100) or (ECL25)
2. Cuvettes (CL011) or (CL010)
3. Wash Reagent (P-594)
4. Trigger SolutionsA and B (P-595)

SPECIMEN COLLECTION AND PREPARATION

Fresh fecal samples should be collected by using a plastic sampling device. The collected fecal sample must be transported, kept at 2-8°C and tested within 2 days. A non-preserved sample must be stored below -20°C for a longer storage period.

Patient samples need to be diluted **1:11** with patient sample diluent before being measured. A 1x working solution of concentrated fecal sample extraction buffer (30669)is suggested in the extraction of samples used with this assay.

1. Label a test tube (12x75mm) or a 2.5 mL plastic vial.
2. Add 2 mL of diluted fecal sample extraction buffer to each tube or vial.
3. Add 200 µL of liquid stool sample to the above tube.
4. With solid stool sample, take an equivalent amount (about 150-200 mg) with a spatula or a disposable inoculation loop. A 6.5 mm inoculation loop may be used to collect and transfer sample into the tube. We suggest to collect twice and then mix well on a vortex mixer.
5. Centrifuge the diluted fecal sample at 1000 rpm (200 g) for 3 minutes. The supernatant can be directly used in the assay. As an alternative to centrifuging, let the diluted samples sit and sediment for 15 minutes and take the clear supernatant for testing.

Note: The supernatant MUST be particle free, debris free and bubble free before loading to the ECL100 machine for accurate results. If necessary, transfer the supernatant into an empty tube to ensure that no particles are present.

CALIBRATION

An active calibration curve is required for all tests. For the assay, calibration is required for the first time use of a reagent lot. Additional calibration is required every 14 days or whenever controls are out of range.

QUALITY CONTROL

The characteristics of patient samples are simulated through controls and are critical to validate the performance of CLIA assays due to the random access format. Use of controls is left to the discretion of the user, based on good laboratory practices, requirements, and applicable laws. We suggest performing a control test once every day. Quality control results that do not fall within acceptable ranges may indicate invalid test results.

ASSAY PROCEDURE

1. Reagents from different kit lot numbers should not be combined or interchanged. Make sure that there are no air bubbles in any reagents, calibrator and control vials.
2. Reagent Preparation

2.1 Remove reagent cartridges from packaging and replace the solid caps with the provided soft caps for ECL100. For ECL25, carefully remove the aluminum foil seal on each container on the cartridges and insert soft caps.

2.2 For the ECL100, take out the Magnetic Particle bottle make sure to roll between hands and gently but thoroughly mix until the magnetic particle solution is homogenous. The solution should be uniform with no clumps of magnetic particles visible; this step is vital for assay performance. For ECL25, mix the magnetic beads by moving back and forth the bottom part of the cartridge at upright position. Make sure to look inside the cartridge until the solution is uniform with no clumps of magnetic particles visible and no air bubbles. Recap the bottle. Open the top soft cap of all reagent bottles, leaving only the hollow soft rubber. The reagents are now ready to be loaded into the ECL100 or ECL 25 for calibration.

3. Assay Program

The following table illustrates the protocol used by the ECL100 or ECL25 for instrument operation.

Component	Quality Control Hole (µL)	Sample Hole (µL)
GDHAntigenControls (L0561 – L0562)	75	-
Extracted Fecal Samples	-	75
Biotin GDHAntibody (L0549)	100	100
GDHMagnetic Particle Solution (L0548)	25	25
Incubation Period 1		
Wash the reaction cup 3 times with the wash reagent.		
Acridinium Ester GDHAntibody (L0550)	100	100
Incubation Period 2		
Wash the reaction cuvette 3 times with wash reagent.		
Trigger Solution A (P-595)	200	200
Trigger Solution B (P-595)	200	200

The assay total incubation time is less than 30 minutes.

INTERPRETATION OF RESULTS

The chemiluminescence analyzer automatically calculates the concentration values of the sample and the control by a standard curve (fitting method: four parameters or point-to-point) and the measured RLU.Control values are compared with the range of the marked value. If it exceeds the indicated quality control range, it indicates that the test is unqualified and needs to be re-tested.

Due to methodological differences or antibody specificity, there may be deviations between the test results of reagents from different manufacturers. Therefore, direct comparisons should not be made to avoid false interpretation.

EXPECTED VALUES

Fecal C. Difficile antigen concentrations were measured in stool samples collected from 45 apparently healthy adults using the EDI™ Fecal Clostridium Difficile GDH Antigen CLIA Kit. The suggested normal cut off is 0.50 ng/mL.

It is highly recommended that each laboratory should establish their own normal range for fecal GDH antigen based on local populations.

LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 or ECL25 Immunoanalyzer only. Refer to the appropriate system manuals for a specific description of installation, start-up, operation, system performance, instructions, calibration, precautions, hazards, maintenance, and troubleshooting.
2. Reagents from different lots cannot be mixed.
3. Test results from this product should not be the sole basis for clinical diagnosis.

4. If the test sample result is higher than the upper limit of the calibration curve, it is recommended to re-measure after dilution according to a certain ratio. The measurement result is recalculated according to the dilution ratio to ensure the accuracy of the result.

PERFORMANCE CHARACTERISTICS

Hook Effect
The assay shows no hook effect up to 250ng/mL.

Limit of Blank
The limit of blank (LoB) was determined by 60 replicates of calibrator matrix to be 0.46 ng/mL.

Limit of Detection
The limit of detection (LoD) was determined by 60 replicates of low-level samples to be 0.51 ng/mL.

Limit of Quantification
The limit of quantification (LoQ) was determined by 60 replicates of low-level samples to be 1.54 ng/mL.

Linearity
Linearity was determined by measuring one diluted standard (L6) of medium GDH antigen concentration. In each assay, the average of tworeplicates of each of the diluted standards is used for a correlation analysis against calculated theoretical values.

Standard L6	Average Measured Concentration (ng/mL)	Theoretical Concentration (ng/mL)	CV (%) Measured Concentration	R^2
10%	7.7	6.25	2%	0.999
20%	14.3	12.5	5%	
40%	30.1	25.0	3%	
60%	44.8	37.5	1%	
80%	58.9	50.0	4%	
100%	76.5	62.5	2%	

Repeatability
Reproducibility was determined by measuring ten replicates of two control samples.

Control	Average Concentration (ng/mL)	CV (%)
3	5.03	3%
8	157.99	4%

Precision
Precision was determined by measuring eight replicates of three control samples a total of three times on three different days.

Control	Average Concentration (ng/mL)	CV (%)
2	2.70	6%
5	19.01	5%
7	82.60	5%

Accuracy
Accuracy was determined by three replicates of two standards used to generate the multi-point calibration curve.

Standards	Measured Concentration (ng/mL)	Average Concentration (ng/mL)	Target Value ± 25% (ng/mL)
L2	3.2	3.11	2.1 – 3.5
	2.4		
	3.7		
L5	19.7	18.65	15.1 – 25.1
	18.4		
	17.9		

WARRANTY
This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCES

1. N. Shetty, M. W.D.Wren, P. G. Coen, The journal of Hospital Infection Hanuary 2011 Colume 77, Issue 1
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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE
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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)