Version 16-04/24

User's Manual

Shiga toxins ELISA Kit

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Shiga toxins ELISA Kit is an enzyme immunoassay for the simultaneous qualitative detection of Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) in a single test. It is intended for use with human fecal samples from patients with gastrointestinal symptoms to aid in the diagnosis of disease caused by Shiga Toxin producing Escherichia coli (STEC). It may be used directly with human fecal specimens, or broth or plate cultures derived from fecal specimens.

General Description

Shiga toxin producing Escherichia coli (STEC) were first described by O' Brien, et al. after discovering that E.coli culture supernatant, which was cytotoxic to HeLa and Vero cells, could be neutralized by rabbit anti Shiga toxin antibodies. STEC cause foodborne and waterborne diarrheal disease worldwide which, if left undiagnosed, can progress to hemorrhagic colitis and/or hemolytic uremic syndrome (HUS). Since certain treatments and medications can increase the risk of HUS, prompt detection is necessary to prevent outbreaks and secondary transmission. STEC strain O157:H7 has historically been the focus of attention in the United States since first isolated from undercooked hamburgers, causing an estimated 73,000 illnesses annually. However, STEC infections caused by non-O157 strains have become more prevalent in recent years, both in the United States as well as abroad. O157:H7 infections are routinely diagnosed by culture of fecal samples on selective media, but this methodology allows nonO157 STEC strains to go undetected. STEC produce either one or both Shiga toxins (Stx1 and/ or Stx2), both potent cytotoxins. Isolates producing only Stx2 have been attributed to higher incidence rates of HUS. Shiga toxins can be detected by tissue culture assay, but this method is both time consuming and labor intensive. By detecting the toxins, the SHIGA TOXIN CHEK test can detect STEC present in fecal samples or culture, regardless of the serotype or other virulence factors.

Principles of Testing

The Shiga toxins ELISA Kit uses antibodies to Stx1 and Stx2. The microassay wells supplied with the kit contain immobilized monoclonal antibodies against Stx1 and Stx2. The detecting antibody consists of a mixture of anti-Stx1 and anti-Stx2 polyclonal antibodies conjugated to horseradish peroxidase. In the assay, an aliquot of a fecal specimen or culture is emulsified in the Diluent and the diluted specimen is then transferred to the microassay well containing the detecting antibody. If Stx1 and/or Stx2 are present in the specimen, they will bind to the detecting antibody and to the immobilized monoclonal antibodies during the incubation phase. Any unbound material is removed during the washing steps. Following the addition of substrate, a color is detected due to the enzyme-antibody-antigen complexes that form in the presence of toxin.

Reagents And Materials Provided

1. Microassay Plate: 12 strips, each strip consisting of 8 wells, coated with monoclonal antibodies specific for Stx1 and Stx2 (stored with desiccant).

2. Diluent (40 mL): buffered protein solution containing 0.02% thimerosal.

3. Substrate (14 mL): solution containing tetramethylbenzidine and peroxide.

4. Wash Buffer Concentrate (50 mL): 20× concentrate containing phosphate buffered saline, detergent, and 0.2% thimerosal.

5. Stop Solution (7 mL): 0.6N sulfuric acid. **CAUTION: Avoid contact with skin; flush with water immediately if contact occurs.**

6. Positive Control (3.5 mL): inactivated antigen in a buffered protein solution containing amphotericin B. Contains 0.05%, ProClin 300.

7. Conjugate (7 mL): polyclonal antibodies specific for Stx1 and Stx2 coupled to horseradish peroxidase in a buffered protein solution containing 0.02% thimerosal.

Materials Required But Not Supplied

1. Wash bottle
2. Timer
3. Vortex
4. Mixer
5. Discard container
6. Distilled water
7. Tubes for dilution of specimen
8. Paper towels or absorbent sheets
9. Applicator sticks
10. Refrigerator set between 2°C and 8°C
11. Incubator set at 37°C ± 2°C
12. Pipetter and tips
13. Disposable gloves for handling fecal samples
14. Swabs or inoculating loops
15. Spectrophotometer capable of reading dual wavelength at 450/620nm or single wavelength at 450nm (a dual wavelength plate reader is recommended; absorbance should be measured at 450nm and referenced at 620nm)

Storage

The kit should be stored between 2°C and 8°C. The kit containing the reagents with designated shelf life should be stored between 2°C and 8°C and should be returned to the refrigerator as soon as possible after use.

Specimen Collection And Preparation

COLLECTION AND HANDLING OF FECAL SPECIMENS:

1. Specimen Handling for Direct Fecal Testing

a. Fresh specimens should be tested as soon as possible after receipt. If testing cannot be performed upon receipt, samples may be stored between 2°C and 8°C or frozen (≤ -10°C) for up to 14 days from sample receipt.

b. Specimens in transport media (C&S or Cary Blair) can be stored between 2°C and 8°C or frozen (≤ -10°C) for up to 14 days from sample receipt.

2. Specimen Handling for Broth or Plate Method

a. Specimens should be stored between 2°C and 8°C and cultured as soon as possible after receipt. If cultures cannot be started within 2 hours of sample receipt, samples may be frozen (≤ -10°C) for up to 14 days from sample receipt.

b. Specimens in transport media (C&S or Cary Blair) can be stored between 2°C and 8°C for up to 5 days.

3. Make sure that specimens are thoroughly mixed before performing the assay.
4. Repeated freeze/thaw cycles should be avoided. If using frozen specimens, thaw at room temperature.
5. If using semi-automated or automated washing equipment, once diluted specimens should be centrifuged (5000 x g for 10 minutes) to remove any particulate matter. If samples cannot be centrifuged, wells must be pre-washed once manually as instructed in the ASSAY PROCEDURE step 11.

SPECIMEN PREPARATION

1. Direct Fecal Specimen Testing (for fresh specimens and samples in transport media)

a. Mix all specimens thoroughly regardless of consistency - it is essential that the specimens be evenly suspended before sampling.

b. Continue to ASSAY PROCEDURE.

2. Broth Method (for fresh specimens and samples in transport media)

a. Mix all specimens thoroughly regardless of consistency - it is essential that the specimens be evenly suspended before inoculating the broth.

Liquid/Semi-solid specimens - transfer 25 µL of specimen into a culture tube containing 5 mL of MacConkey or 8 mL of Gram-Negative (GN) broth. Vortex for 10 seconds.

Formed/Solid specimens - transfer a small portion (approximately 2 mm diameter, the equivalent of 25 µ L) of the specimen into a culture tube containing 5 mL of MacConkey or 8 mL of GN broth.

Fecal specimens in Cary Blair or C&S transport media - transfer 100 µL of the preserved specimen to a culture tube containing 5 mL of MacConkey or 8 mL of GN broth.

b. Loosely cap the inoculated broth tubes and incubate for 16-24 hours between 35°C and 39°C.

c. Examine the tube for growth. If there is no growth, do not proceed with testing. Instead, inoculate another tube of broth with either the same fecal specimen or a fresh specimen from the same patient. Alternatively, the selective plate method (see "3" below) or direct fecal specimen testing method (see "A" above) may be used.

d. Continue to ASSAT PROCEDURE.

3. Plate Method (for fresh specimens and samples in transport media)

a. Mix all specimens thoroughly regardless of consistency - it is essential that the specimens be evenly

suspended before inoculating the plate. Use a swab to sample the specimen, and then spread on a SMAC, CT-SMAC, or CHROMagar® O157 plate. NOTE: CT-SMAC and CHROMagar® O157 are more selective than SMAC plates and may inhibit the growth of non-O157 STEC.

b. Incubate the plates for 16-24 hours between 35°C and 39°C.

c. Examine the plate for growth. If there is no growth, do not proceed with testing. Instead, inoculate another plate with either the same fecal specimen or a fresh specimen from the same patient. Alternatively, the broth method (see "2" above) or direct fecal specimen testing method (see "1" above) may be used.

d. Continue to ASSAY PROCEDURE.

Reagent Preparation

1. All reagents must be at room temperature prior to use in the assay.
2. Prepare 1× Wash Solution. The Wash Buffer Concentrate is supplied as a 20× concentrate (a precipitate may be noticed). It should be diluted to a total volume of 1 liter by adding 50 mL of the concentrate to 950 mL of distilled water. Label the bottle. Store any unused 1X Wash Solution between 2°C and 8°C.
3. Assay Strip Preparation. Each strip contains 8 wells coated with monoclonal antibodies speciffc for Stx1 and Stx2. Each specimen or control will use one of these coated wells. Determine the number of wells to be used. Avoid contact with the base of the wells. Assay wells not used must be returned to the plastic bag and carefully resealed with desiccant.

Assay Procedure

1. Bring all reagents and the required number of test strips to room temperature before use.
2. Set up and label one test tube for each sample as necessary.
3. Add Diluent to each tube using a transfer pipette. The pipettes have raised graduations at 50 µL, 100 µL, 200 µL and 300 µL.
4. Obtain one disposable plastic transfer pipette (supplied with the kit) for each sample.
5. Mix all specimens and cultures thoroughly regardless of consistency- it is essential that the samples be evenly suspended before sampling. Add the required amount of specimen or culture to the tube.

   Direct fecal testing - Liquid/Semi-solid specimens - sample 50 µL of specimen with a transfer pipette and dispense into the Diluent tube. Use the same transfer pipette to mix the diluted specimen. If testing cannot be performed immediately after dilution, samples may be stored between 2°C and 8°C for up to 2 days.

   Direct fecal testing - Formed/Solid specimens – care must be taken to add the correct amount of formed feces to the sample mixture. Mix the specimen thoroughly using a wooden applicator stick and transfer a small portion (approximately 3 mm diameter, the equivalent of 50 µL) of the specimen into the Diluent tube. Emulsify the specimen using the applicator stick. If testing cannot be performed immediately after dilution, diluted samples may be stored between 2°C and 8°C for up to 2 days.

   Fecal specimens in transport media (Cary Blair or C&S) – sample 100 µL of specimen with a transfer pipette and dispense into the Diluent tube. Use the same transfer pipette to mix the diluted specimen.

   Broth cultures – sample 50 µL of specimen with a transfer pipette and dispense into the Diluent tube. Use the same transfer pipette to mix the diluted specimen.

Plate cultures –sweep through a confluent area on the plate several times or pick individual colonies with an inoculating loop, then mix the loop in the Diluent tube. Rotate the loop against the inside of the tube several times to release the sample and remove the loop.

NOTE: Transferring too little sample, or failure to mix and completely suspend the sample in the Diluent, may result in a false-negative test result.

6. Close each tube of diluted sample or control and mix thoroughly. Proper mixing can be achieved by vortexing or inverting the tube several times.
7. Add 1 drop (50 µL) of Conjugate (red cap) to each well. Be sure to hold each bottle vertically when adding the drops. Use 1 well for each fecal specimen, 1 well for the Positive Control and 1 well for the negative control. Identification marks may be written directly on side of well.
8. Using a new transfer pipette, transfer 100 µL of diluted specimen to the assay well. Add 1 drop (50 µL) of the Positive Control (black cap) to the positive control well and 100 µL of the Diluent (negative control) to the negative control well. Tap the sides of the plate to mix.
9. Cut the adhesive plastic sheet to the size necessary to cover the wells. Cover the wells and incubate them at 37°C ± 2°C for 50 minutes.
10. Shake out the contents of the assay wells into a discard pan.
11. Wash each well using the 1× Wash Solution in a squirt bottle with a fine-tipped nozzle, directing the Wash Solution to the bottom of the well with force. Fill the wells, and then shake the Wash Solution out of the well into a discard pan. Slap the inverted plate on a dry paper towel. **Note: If using semi-automated or automated washing equipment, add 350 µL of 1× Wash Solution to each well. Wash for a total of 5 times (4 times if performing a manual pre-wash for non-centrifuged samples).**
12. Repeat step 11 four additional times using a dry paper towel each time. If any particulate matter is seen in the wells, continue washing until all the particulate matter is removed.
13. After washing, completely remove any residual liquid in the wells by striking the plate once again onto a dry paper towel until no liquid comes out. Dispose of paper towels and specimen containers properly.
14. Add 2 drops (100 µL) of Substrate (blue cap) to each well. Gently tap the wells to mix the substrate. Incubate the wells at room temperature for 10 minutes. Gently tap the wells at 5 minutes.
15. Add 1 drop (50 µL) of Stop Solution (yellow cap) to each well. Gently tap the wells and wait 2 minutes before reading. The addition of the Stop Solution converts the blue color to a yellow color which may be quantitated by measuring the optical density at 450 nm on a microplate ELISA reader. The instrument should be blanked against air. If a dual wavelength reader is used, blank against air at 620 nm and read at 450 nm. Wipe the underside of each well before measuring the optical density. If an ELISA reader is unavailable, the test may be read visually in good light against a white background. Read within ten minutes after adding Stop Solution.

Quality Control

1. A positive and negative control must be run with each series of test specimens.
2. Positive and negative controls must fall within their respective ranges or the test is not valid.

1) Positive Control must be a visible yellow color. If read on a spectrophotometer, the OD at 450 nm or using dual wavelength at 450/620 nm must be ≥ 0.500.
2) Negative Control must be visually clear. If read on a spectrophotometer, the OD at 450 nm must be < 0.120. If read at 450/620 nm the absorbance must be < 0.080.

3. Wells that are clear visually but give absorbance ≥ 0.120 OD at 450 nm should be wiped on the underside and re-measured.
4. Visual readings must be taken in good light against a white background.
5. A sample that yields a weak positive result (i.e., < 0.200) and is adjacent to a strong positive should be repeated to assure carryover did not occur.

Interpretation Of Results

A positive test result indicates that Stx1 and/or Stx2 are present in the specimen. A negative result indicates that Stx1 and/or Stx2 are absent or the level is below the detection limit of the test.

Note: Due to the epidemiological importance of obtaining Shiga toxin positive bacterial isolates, it is recommended that all toxin positive samples undergo bacterial culture to isolate the toxin producing organisms. It is suggested that laboratories perform bacterial culture on all positive samples or coordinate the process with their local & state health laboratories in the United States.

Reference Values

The Shiga toxins ELISA Kit detects the presence of Stx1 and Stx2. Expected values for a particular population should be established by each laboratory. The positivity rate may be dependent upon a number of factors including geography, process of specimen collection, handling and transport, patient age.

Shiga toxin E. coli is the source of an estimated 110,000 cases (0.04% of the population) of foodborne illness annually in the United States. Reported incidence rates in fecal samples submitted for testing range from 0% - 4.1% and vary depending upon the season, geographical location, and patient population, with higher incidence rates seen in the summer months and in preschool-aged children and the elderly. A positive result in the Shiga toxins ELISA Kit confirms the presence of Shiga toxin in the sample; a negative result indicates the absence of toxin or insufficient levels of toxin for detection.

Performance Characteristics

Direct Fecal Testing

The performance of the SHIGA TOXIN test was compared to the Vero Cell Cytotoxin Assay (with neutralization), considered the clinical reference standard (gold standard), and included 899 fresh and 14 frozen specimens. Age information was available for 902 patients. Of the 902 patients, 8.8% were ≤ 18 years. The following table shows a summary of the clinical performance of the SHIGA TOXIN test. The results show that the test exhibited a sensitivity of 100% a speciffcity of 99.9% and an overall correlation of 99.9% with the cytotoxin assay.

Direct Fecal Testing Results

Broth Cultures

The performance of the SHIGA TOXIN test using overnight broth cultures (GN or MacConkey broth) from fecal specimens was compared to the Vero Cell Cytotoxin Assay (with neutralization), considered the clinical reference standard (gold standard). The following table shows a summary of the clinical performance of the SHIGA TOXIN test. The results show that the test exhibited a sensitivity of 97.1%, a speciffcity of 99.7% and an overall correlation of 99.5% with the cytotoxin assay.

Broth Culture Testing Results

Precision

Intra-Assay

For the determination of intra-assay performance, 6 positive fecal specimens and 6 negative fecal specimens were analyzed. Each specimen was assayed in replicates of eight. All positives remained positive and all negatives remained negative.

Inter-Assay

The inter-assay precision of the Shiga toxins ELISA Kit was determined using 12 fecal specimens (six negative, two positive for Stx1, two positive for Stx2, and two positive for both Stx1 and Stx2). The samples were tested, twice a day over a 5-day period using 2 different kit lots. A positive and negative control was run on each day. All positives remained positive and all negatives remained negative.

Sensitivity

The cutoff for the test for direct fecal specimens was established at concentrations of 0.28 ng/mL Stx1 and 0.23 ng/mL Stx2, and for broth cultures at concentrations of 0.18 ng/mL Stx1 and 0.30 ng/mL Stx2.

Specificity

The SHIGA TOXIN test was evaluated for cross-reactivity with the bacterial and viral strains listed below. None of the strains were shown to interfere with the performance of the SHIGA TOXIN test.

Aeromonas hydrophila, Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, Candida albicans, Citrobacter freundii, Clostridium difffcile, Clostridium perfringens, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli, Escherichia coli O157:H7 (non-toxigenic), Escherichia coli EIEC (enteroinvasive), Escherichia coli EPEC (enteropathogenic), Escherichia coli ETEC (enterotoxic), Escherichia fergusonii, Escherichia hermannii, Gardnerella vaginalis, Helicobacter pylori, Klebsiella pneumoniae, Lactobacillus acidophilus, Proteus vulgaris, Providencia stuartii, Pseudomonas aeruginosa, Pseudomonas ffuorescens, Salmonella enteric serovar minnesota, Salmonella typhimurium Serratia liquefacians, Shigella ffexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus aureus (Cowan), Staphylococcus epidermidis, Yersinia enterocolitica, Human Adenovirus, Type 2, 14, 40 and 41, Human Coxsackievirus A9, B1, Human Enterovirus 69, Feline calicivirus, Human rotavirus

Reproducibility

The reproducibility of the SHIGA TOXIN test was determined using 11 fecal specimens that were coded to prevent their identiffcation during testing. Testing was performed at 2 independent laboratories and on-site at CD. The samples were tested, twice a day over a 5-day period by multiple technicians at each site using 2 different kit lots. A positive and negative control was run with each panel of the masked samples. The results from each laboratory were submitted to CD. and compared with in-house results. The results were consistent among the different locations, and exhibited a correlation of 100%. The samples produced the expected results 100% of the time.

Precautions

1. Each component in the kit should be inspected for any signs of leakage. Upon arrival, inspect the kit to ensure that components are not frozen or warm to the touch due to improper shipping conditions.
2. Reagents from different kits should not be mixed or interchanged. Do not use a kit past the expiration date.
3. Caps and tips are color-coded; do NOT mix or interchange!
4. Bring all components to ROOM TEMPERATURE BEFORE USE!
5. Do not freeze the reagents. The kit should be stored between 2°C and 8°C.
6. When handling assay wells, avoid scratching the bottom of the wells because this may result in elevated absorbance readings.
7. Unused microwells must be placed back inside of the resealable pouch with the desiccant to protect them from moisture. Check the desiccant pack before using the microwells. The color indicator on the desiccant pack should be blue. If the color turns pink, the quality of the microwells may be compromised. Please do not use microwells stored with pink desiccant.
8. Hold reagent bottles vertically to dispense reagents to ensure consistent drop size and correct volume.
9. Handle specimens and used microassay wells as if capable of transmitting infectious agents. Wear gloves when performing the test.
10. Use fecal specimens within 24 hours of collection to obtain optimal results.
11. Reagents contain 0.02% thimerosal as a preservative and should be handled with normal laboratory caution.
12. Microbial contamination of reagents may decrease the accuracy of the assay. Avoid microbial contamination of reagents by using sterile disposable pipettes if removing aliquots from reagent bottles.
13. The Stop Solution contains 0.6N sulfuric acid. Flush with water immediately if contact occurs.
14. Perform the washing procedure as directed to avoid high background reactions.
15. The Substrate is light sensitive and should be protected from direct sunlight or UV sources.
16. The test has been optimized for sensitivity and specificity. Alterations of the specified procedure and/or test conditions may affect the sensitivity and specificity of the test. Do not deviate from the specified procedure.
17. Fecal specimens may contain potentially infectious agents and should be handled at "Biosafety Level 2" as recommended in the CDC/NIH Manual "Biosafety in Microbiological and Biomedical Laboratories."

Limitations

1. The Shiga toxins ELISA Kit is used to detect Stx1 and Stx2 in fecal specimens and cultures derived from fecal specimens. The test confirms the presence of Stx1 and/ or Stx2 in the sample, and this information should be taken under consideration by the physician in light of the clinical history and physical examination of the patient.
2. A negative test result does not preclude the possibility of the presence of Shiga toxins in the specimen which may occur if the level of antigen is below the detection limit of the test.
3. The Shiga toxins ELISA Kit is qualitative. The intensity of the color should not be interpreted quantitatively.
4. The toxin produced by Shigella dysenteriae is nearly identical to Shiga Toxin 1 produced by E. coli and if present at detectable levels, will give a positive result in the well.