ECO-F23

E. coli O157 Antigen Rapid Test Device (Feces)

INTENDED USE

The *E. coli* O157 Antigen Rapid Test Device is an *in vitro* immunoassay for the direct and qualitative detection of antigens from shiga-toxigenic *E. coli* O157. It is intended to aid in the rapid diagnosis of *E. coli* O157 infection. Human fecal specimens can be used directly by the test. Negative results do not preclude *E. coli* O157 infection and should be confirmed via culture or molecular method. The test is for professional use only.

INTRODUCTION

Escherichia coli is a gram-negative, facultatively anaerobic bacterium of the genus *Escherichia* commonly found in the lower intestine of endotherms as normal gut flora[1].While many different serotypes have been identified, certain strains of *E. coli* have acquired genes for toxins from other bacterial species via bacteriophage plasmids. Shiga-toxigenic groups of *E. coli* (STEC) are food-borne pathogens that cause gastrointestinal illness, hemorrhagic colitis, hemolytic uremic syndrome (HUS) and death, although infection can be asymptomatic. Morbidity and mortality rates in children, infants and the elderly are especially high[2].

E. coli O157: H7 is one of the STEC serotypes most often implicated in outbreaks[3].The pathogens can spread by fecal-oral route, and have also been traced to contaminated raw leaf green vegetables, water and undercooked meat. Transmission is via the fecal-oral route, and most illness has been through distribution of contaminated raw green vegetables and undercooked meat[4]. Most sorbitol-nonfermenting *E. coli* O157 are motile and possess the H7 antigen. However motility can be difficult to elicit, resulting in failure to detect the H7 antigen. Besides, a sorbitol-nonfermenting strain of *E. coli* O157 is occasionally either nonmotile or motile with a nontypable flagellar antigen (O157: H-). Therefore, H7 antigen determination is not necessary for clinical laboratory evaluation of a strain of *E. coli* O157: H7[5].

Diagnostic methods for *E. coli* O157 include culture on Sorbitol-MacConkey (SMAC) agar followed by confirmatory testing[6]. However, culture diagnosis is time-consuming. Swifter diagnosis is possible using quick *E. coli* DNA extraction plus PCR techniques which is often an expensive method and requires well-trained personnel and sophisticated equipment[7]. Latex agglutination test is also feasible, and antibody detection is also feasible under development. Meanwhile, owing to the simplicity and rapidity, enzyme immunoassay (EIA) and immnunochromatography for either O157 antigens or toxins could be valuable during outbreak investigations. The *E. coli* O157Antigen Rapid Test Device is a qualitative, lateral flow immunoassay for the detection of shiga-toxigenic *E. coli* O157 in human fecal specimens. The test is specific to shiga-toxigenic *E. coli* O157 antigens with no known cross-reactivity to normal flora or other intestinal pathogens.

PRINCIPLE

The *E. coli* O157 Antigen Rapid Test Device detects *E. coli* O157 antigens through visual interpretation of color development. Anti-*E. coli* O157 antibodies are immobilized on the test region (T) of the membrane. A fecal sample is added to the sample diluent buffer which is optimized to extract the *E. coli* O157 antigens from specimen. During testing, the extracted antigens, if present, will bind to anti-lipopolysaccharide (LPS) antibodies conjugated to colored particles on the sample pad. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by anti-*E. coli* O157antibodies at the detection zone. Excess colored particles are captured at the internal control zone.

The presence of a colored band in the test region (T) indicates a positive result for the particular antigens, while its absence indicates a negative result. A colored band at the control region (C) serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking is working.

MATERIALS

Materials Provided

- Individually packed test devices
- Package insert
- Specimens dilution tube with buffer

Materials Required but Not provided

- Centrifuge
- Specimens collection container
- Dropper
- Clock, timer or stopwatch
- Disposable latex gloves

PRECAUTIONS

- For *in vitro* Diagnostic Use Only.
- Read the Package Insert prior to use. Directions should be read and followed carefully.
- Do not use kit or components beyond the expiration date.
- The device contains material of animal origin and should be handled as a potential biohazard. Do not use if pouch is damaged or open.
- Test devices are packaged in foil pouches that exclude moisture during storage. Inspect each foil pouch before opening. Do not use devices that have holes in the foil or where the pouch has not been completely sealed. Erroneous result may occur if test reagents or components are improperly stored.
- Do not use the Sample Diluent Buffer if it is discolored or turbid. Discoloration or turbidity may be a sign of microbial contamination.
- Patient specimens and used *E. coli* O157 Antigen Rapid Test device may contain infectious agents and should be handled and discarded as if they are biologically hazardous. All specimens must be mixed thoroughly before testing to ensure a representative sample prior to testing.
- Care should be taken to store specimens as indicated in the document.
- Any deviation below or above set incubation times may affect sensitivity and specificity and should be avoided.
- Failure to bring specimens and reagents to room temperature before testing may decrease assay sensitivity. Inaccurate or inappropriate specimen collection, storage, and transport may yield false negative test results.

STORAGE AND STABILITY

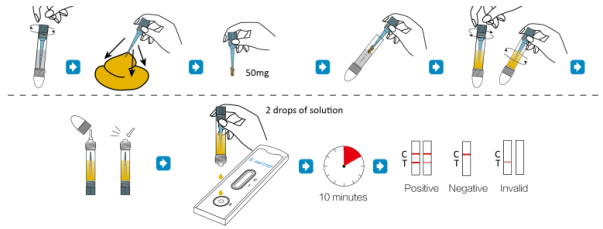
- Store the *E. coli* O157 Antigen Rapid Test Device at 2~30ºC when not in use.
- **DO NOT FREEZE**.
- Kit contents are stable until the expiration dates marked on their outer packaging and containers.
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipments, containers or reagents can lead to false results.

TEST PROCEDURE

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) before use.

1. Specimen collection and pre-treatment:
   1) Use clean, dry specimen containers for specimen collection. Best results will be obtained if the assay is performed within 2 hours after collection.
   Note: Specimens collected in the specimen container may be stored for 1~2 days at 2~8ºC or for 3 months at -20°C.
   2) **For solid specimens:** Unscrew and remove the dilution tube applicator. Be careful not to spill or spatter solution from the tube. Collect specimens by inserting the applicator stick into at least 3 different sites of the feces to collect approximately 50 mg of feces (equivalent to 1/4 of a pea).
   For liquid specimens: Hold the pipette vertically, aspirate fecal specimens, and then transfer approximately 100uL of the liquid specimen into the sample diluent tube.
   3) Place the applicator back into the tube and screw the cap tightly. Be careful not to break the tip of the dilution tube.
   4) Shake the specimen collection tube to mix the specimen and the diluent buffer thoroughly.
2. Testing
   1) Remove the test device from its sealed pouch, and place it on a clean, level surface. Label the test with patient or control identification. To obtain a best result, the assay should be performed within 2 hours.
   2) Using a piece of tissue paper, break the tip of the dilution tube. Hold the tube vertically and dispense 2 drops of solution into the specimen well (S) of the test device.
   Avoid trapping air bubbles in the specimen well (S), and do not drop any solution in observation window.
3. Wait for the colored band(s) to appear. The result should be read at 10 minutes. Do not interpret the result after 20 minutes.

Note: If the specimen does not migrate (presence of particles), centrifuge the specimens contained in the sample diluent tube. Collect 100 µL of supernatant, dispense into the specimen well (S) of a new test device and start afresh following the instructions mentioned above.



INTERPRETATION OF RESULTS

POSITIVE: Two colored bands appear on the membrane. One colored band appears in the control region (C) and one colored band appears in the test region (T).

NEGATIVE: Only one colored band appears, in the control region (C). No apparent colored band appears in the test region (T).

INVALID: Control band fails to appear. Results from any test which has not produced a control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of color in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region should be considered positive. Note that this is a qualitative test only, and cannot determine the concentration of analytes in the specimen.
2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

- The *E. coli* O157 Antigen Rapid Test Device has built-in (procedural) controls. Each test device has an internal standard zone to ensure proper sample flow. The user should confirm that a colored band located at the "C" line is present before reading the result.
- Good laboratory practice suggests testing positive and negative external controls to ensure that the test reagents are working and that the test is correctly performed.

LIMITATIONS OF THE TEST

1. The *E. coli* O157 Antigen Rapid Test Device is for professional *in vitro* diagnostic use, and should only be used for the qualitative detection of shiga-toxigenic *E. coli* O157. The intensity of color in a positive band should not be evaluated as "quantitative or semi-quantitative".
2. Both viable and nonviable *E. coli* O157 bacteria are detectable with the *E. coli* O157 Antigen Rapid Test Device.
3. As with all diagnostic tests, a definitive clinical diagnosis should not base on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
4. Failure to follow the TEST PROCEDURE and INTERPRETATION OF RESULTS may adversely affect test performance and/or invalidate the test result.
5. Results obtained with this assay, particularly in the case of weak test lines that are difficult to interpret, should be used in conjunction with other clinical information available to the physician.
6. A high dose "hook effect" may occur where the color intensity of test band decreases as the concentration of antigen increases. If a "hook effect" is suspected, dilution of specimens may increase color intensity of the test band.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity (limit of detection)

The limit of detection of *E. coli* O157 Antigen Rapid test is 2 x 10^4 CFU/mL for *E. coli* O157.

Clinical sensitivity and specificity

413 patient fecal samples were collected and tested on the *E. coli* O157 Antigen Rapid Test and a commercial *E. coli* O157 antigen rapid test. Comparison for all subjects is shown in the following table:

	***E. coli* O157Antigen Rapid Test**		
Reference	**Positive**	**Negative**	**Total**
Positive	152	0	152
Negative	1	260	261
Total	153	260	413

Relative Sensitivity: ＞99.9% (97.5%~100%)*
Relative Specificity: 99.6% (97.9%~99.9%)*
Overall Agreement: 99.8% (98.6%~100%)*
***95% Confidence Interval**

Cross Reactivity

Cross reactivity with following organisms has been studied at 1.0 x 10^9 organisms/mL. The following organisms were found negative when tested with the *E. coli* O157 Antigen Rapid Test Device (Feces).

Salmonella spp	*Campylobacter* spp	*Shigella* spp
Helicobacter pylori	*Enterococcus faecalis*	*Enterococcus faecium*
Norovirus	*Proteus mirabilis*	*Clostridium difficile*
Staphylococcus aureus	*Rotavirus*	*Adenovirus*
Pseudomonas aeruginosa	*Neisseria meningitidis*	*Neisseria gonorrhea*
Group C Streptococcus	*Gardnerella vaginalis*	*Group B Streptococcus*
Klebsiella pneumoniae	*Candida albicans*	

LITERATURE REFERENCES

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4. Manley WA, Walford JR, Harrison JF, McKee GL. ImmunoCard STAT! (ICS) *E. coli* O157:H7 test in a suspect waterborne outbreak in Wyoming. ASM, Abstract submitted for 1999 General Meeting. 1998.
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GLOSSARY OF SYMBOLS

ρ	Catalog number	0	Temperature limitation
ι	Consult instructions for use	A	Batch code
I	In vitro diagnostic medical device	ε	Use by
µ	Manufacturer	T	Contains sufficient for <n> tests
σ	Do not reuse	A	Authorized representative in the European Community
Y	CE marking according to IVD Medical Devices Directive 98/79/EC		

µ
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