STANTON TERRITORIAL HEALTH AUTHORITY

Yellowknife, Northwest Territories

TITLE: E. coli O157	**Revision Date:** 20-April-2018	**Issue Date:** 20-April-2016
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Distribution: Microbiology Test Manual	**Page:** 1 of 5	
Approved by: S. Asmussen, Manager of Diagnostic Services	**Signed by:** 	

PURPOSE:

Rapid identification kit intended for the confirmatory identification of *Escherichia coli* serogroup O157 cultured on selective solid media from human fecal samples. The test allows for the rapid differentiation of *E.coli* O157 from other non-sorbitol fermenting organisms.

Latex particles are coated with antibodies against the somatic lipopolysaccharide O157 antigen of *E.coli* O157. When latex particles are mixed with a suspension containing *E.coli* O157 antigens, agglutination will occur.

SAMPLE INFORMATION:

Type	Non-sorbitol fermenter
Source	Sorbitol MacConkey plate, 18-24 hours old

REAGENTS and/or MEDIA:

The following reagents are included in the Microgen® *E.coli* 0157 (REF:M44) kit and should be stored at 2-8°C:

- M44a - Reagent Test Latex (blue cap)
- M44b - Reagent Control Latex (black cap)
- M40 - 0.85% Isotonic Saline (white cap)

SUPPLIES:

- Disposable agglutination cards
- Disposable mixing sticks

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

Sodium azide is used as a preservative in this kit and can react with lead and copper plumbing to form potentially explosive metal azides. Dispose by flushing with large amounts of water to prevent azide buildup.

QUALITY CONTROL:

A QC order will be automatically generated in the TQC system:

- Resulting Worklist→MICS→O157

Perform the following QC with each test:

Step	Action
Performing the Reagent Control	
1	Add 1 drop of Test Latex(M44a) to a well on the agglutination slide
2	Add 1 drop of Control Latex (M44b) to a well on the agglutination slide
3	Add 1 drop of saline solution (M40) to each drop of latex and mix each latex/saline suspension – spreading the liquid over the entire surface of the well
4	Rock the card gently for 30 seconds and observe for agglutination
5	If agglutination is observed, then either the latex or the saline is giving non-specific agglutination and should be discarded

Step	Action
Performing The Positive Control	
1	Dispense one drop of saline (M40) each to two separate wells on the agglutination card
2	Using a disposable stick, prepare a smooth suspension of *E. coli* ATCC43888 in the saline drop on both wells
3	Rock the slide gently for 30 seconds and observe for agglutination
4	If no agglutination is observed, add 1 drop of Test Latex (M44a) to one well
5	Add 1 drop of Control latex(M44b) to the other well
6	Rock the slide gently for 2 minutes and observed for agglutination
7	The well containing the Test Latex (M44a) should show agglutination
8	The well containing the Control Latex (M44b) should NOT show agglutination
9	If this reaction pattern has not occurred, the reagents should be discarded

PROCEDURE INSTRUCTIONS:

Step	Action
Performing E. coli 0157 Testing	
1	Dispense one drop of saline (M40) each to two separate wells on the agglutination card
2	Using a wooden stick, remove several well-isolated non-sorbitol fermenter colonies

3	Emulsify the colonies into the 2 drops of saline on the agglutination card to produce a heavy, smooth suspension
4	Spread the suspension over the entire well
5	Rock the slide gently for 30 seconds and observe for agglutination – if the suspension is smooth proceed with the test (see step 6) If the suspension is "stringy" or "granular" the sample is unsuitable for testing and proceed with a VITEK GNI
6	Gently shake the Test Latex and Control Latex to ensure a homogeneous suspension
7	Add 1 drop of Test Latex to one of the wells on the agglutination card. Do not allow the dropper to come in contact with the bacterial suspension
8	Add 1 drop of Control Latex to the remaining well on the agglutination card. Do not allow the dropper to come in contact with the bacterial suspension
9	Using separate wooden sticks mix the suspensions. Discard the application sticks into the AccelTB discard container.
10	Rock the cards gently for 2 minutes and observe for agglutination
11	Discard the agglutination cards in the AccelTB discard container

INTERPRETATION OF RESULTS:

IF	THEN
Test Latex: Agglutination **Control Latex:** No Agglutination	**Presumptive Positive** for: *E.coli* O157 Confirm with a VITEK GNI
Test Latex: No Agglutination **Control Latex:** No Agglutination	**Negative:** *E.coli* O157
Test Latex: Agglutination **Control Latex:** Agglutination	Non-specific agglutination occurred Perform VITEK GNI
Test Latex: No Agglutination **Control Latex:** Agglutination	Inconclusive Result Perform VITEK GNI

REFERENCES:

- Microgen BioProducts. (n.d.). M44 Microgen E.coli O157.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31Dec13	Initial Release	Darrach (A)
2.0	31Mar16	Update of "Special Safety Precautions" to reflect risk assessment recommendations.	C. Russell