

**Procedure:** Ecolex™ 0157

Prepared by	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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**PRINCIPLE:**

The Ecolex 0157 test will identify *E. coli* strains possessing the 0157 serogroup antigen. The test is best used in conjunction with Sorbitol MacConkey Agar. *E. coli* 0157:H7 strains do not ferment Sorbitol and therefore give colorless colonies on this medium. The majority of *E. Coli* isolates ferment Sorbitol and give characteristic pink colonies.

Non-sorbitol fermenting colonies can be tested with the Ecolex 0157 reagents, to determine whether the isolate belongs to the 0157 serogroup and therefore a potential VT-producing strain.

**SPECIMEN:**

**Type:**

Non-sorbitol fermenting (NSF) colonies may be taken from Sorbitol MacConkey Agar or alternatively NSF isolates may be inoculated onto other suitable media.

**EQUIPMENT AND MATERIALS:**

Materials Provided

- Test Reagent
- Control Reagent
- Positive Control Suspension
- Negative Control Suspension
- Disposable Reaction Cards
- Package Insert

Materials required but not provided

Pasteur pipettes  
Microbiological loop  
0.85% saline  
Mixing sticks

**Storage Requirements:**

2-8°C

**QUALITY CONTROL:**

One drop of the positive and negative control suspensions provided should be used in place of the saline/colony suspension described in Step 4 to check the correct performance of the test and control reagents each day Ecolex 0157 is used. Vigorously resuspend the positive and negative control suspensions before use.

The positive control suspension must cause visible agglutination with the test reagent within one minute.

The negative control suspension should cause no agglutination with the test reagent within one minute.

Do not use the test if reactions with the control suspensions are incorrect.

**PROCEDURE - STEPWISE:**

1. Bring the reagents to room temperature. Make sure the test and control reagents are mixed by shaking. Expel any latex from the dropper for complete mixing.
2. Dispense 1 drop of the test reagent close to the edge of a circle on the disposable reaction card.
3. Add a drop of saline to the same circle. Ensure that the test reagent and saline do not mix at this stage.
4. Pick off a portion of a colony to be tested and carefully emulsify it in the drop of saline on the card. Ensure that the resulting suspension is smooth.
5. Mix the test reagent and suspension together and spread to cover the entire area of the circle.
6. Rock the card in a circular motion for up to 1 minute and observe for agglutination under normal lighting conditions. Do not use a magnifying glass.
7. If no agglutination occurs then proceed to test other NSF colonies if they are present.
8. If agglutination with the test reagent does occur, then it is necessary to test a further portion of the colony with the control reagent to ensure that the isolate is not an auto-agglutinating strain. Repeat steps 2 through to 6 substituting the control reagent for the test reagent and test the remaining portion of the colony that agglutinated with the test reagent.
9. Dispose of the used reaction cards properly.

**REPORTING RESULTS:**

Positive Result

Agglutination of the test reagent within one minute is a positive result. This indicates the presence of *E. coli* 0157.

Negative Result

No agglutination occurring within one minute is a negative result. This indicates the absence of *E. coli* 0157.

Uninterpretable

Results cannot be interpreted if there is agglutination of both the test and control reagents. Some strains of *E. coli* are difficult to emulsify in saline and may give a stringy type reaction with both the test and the control reagents.

This should be ignored. If the stringiness is too severe for a correct judgment to be made then the colony should be suspended in 0.3ml of saline. Allow the lumps to settle and test one drop of the smooth supernatant with one drop of the test or control reagent on the reaction card.

### **PROCEDURE NOTES:**

After use, return the kit to the refrigerator ensuring that the bottles are stored in an upright position and the caps are secured.

### **LIMITATIONS OF THE PROCEDURE:**

It may be necessary to test up to 10 separate NSF colonies to ensure a high probability of detecting any 0157 strains which may be in mixed culture with other NSF *E. coli* serotypes.

The use of the control reagent will ensure that the isolate is not an autoagglutinating strain.

If a positive result is obtained on a colony not resembling *E. coli*, then biochemical tests should be performed to confirm that the organism is an *E. coli* strain. Neither the Sorbitol MacConkey Agar nor the Ecolex 0157 will directly confirm an isolate as a toxin producing strain.

Strains of *Escherichia hermannii* may cross react with *E. coli* 0157 sera due to a shared antigen (Borczyk et al)<sup>1</sup>. *E. hermannii* may be differentiated from *E. coli* by the former's fermentation of cellobiose, growth in the presence of KCN, negative reaction in the 4-Methylumbelliferone glucuronide (MUG) assay and yellow pigmentation, which may be delayed.

### **REFERENCES:**

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