Method for enumerating *Escherichia coli* O157:H7 in compost and aged manure

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1. Ten g of a compost or aged/stockpiled manure sample is mixed with 90 mL of tryptic soy broth (TSB; per liter: 30 g) or other appropriate diluent such as buffered peptone water in a sterile filtered sample bag. The sample and diluent can be mixed by massaging the bag by hand, or by stomaching if there are no rocks or other sharp objects in the sample.

   (If the sample will be further enriched for isolation of low numbers of the pathogen, dilution in most other enrichment media is appropriate. For enrichment of samples containing fresher manure, we typically use phosphate-buffered TSB [per liter: 30 g TSB, 2.31 g KH_2PO_4, 12.54 g K_2HPO_4, pH 7.2].)

2. A one-mL volume of the mixture is removed to a sterile 1.5 mL microcentrifuge tube, which is vortexed and allowed to set for 2-3 minutes for debris to settle.

3. Using a Spiral Plater, a 50 µL volume of the sample is plated onto CHROMagar O157 (DRG International) containing 5 mg/L novobiocin and 2.5 mg/L potassium tellurite (ntCHROMagar O157).

4. The ntCHROMagar O157 plates are incubated at 42ºC for 20 to 24 hours and enumerated. *E. coli* O157 colonies are flat, mauve-colored colonies without distinct centers. Colonies are tested for agglutination with *E. coli* O157 latex test reagents or with DrySpot *E. coli* O157 tests.

5. Agglutination-positive colonies are streaked for isolation and further confirmed as *E. coli* O157 by PCR as described by Hu et al., 1999.

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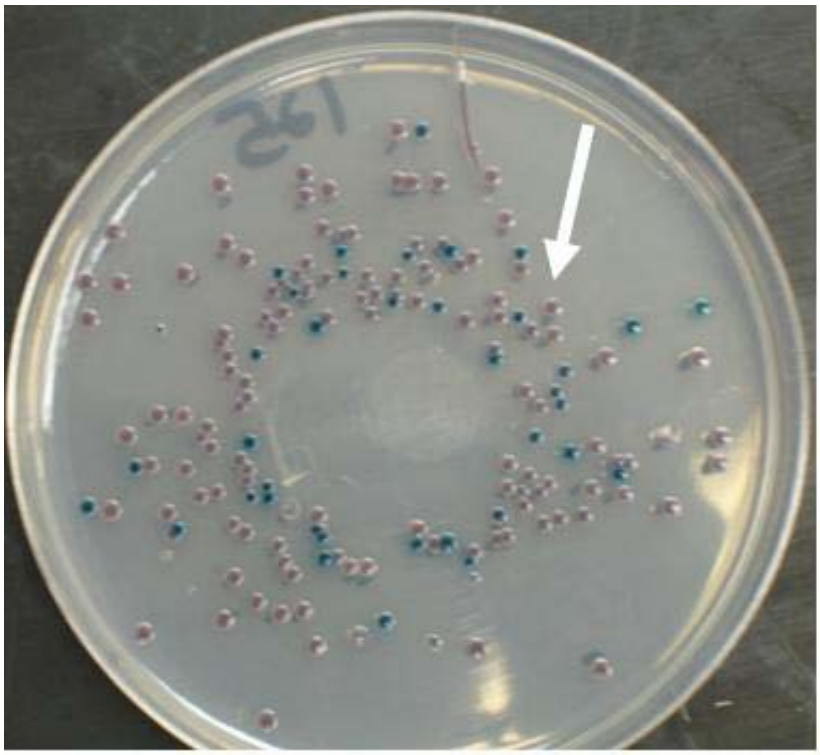

Figure 1. *E. coli* O157:H7 on ntCHROMagar O157

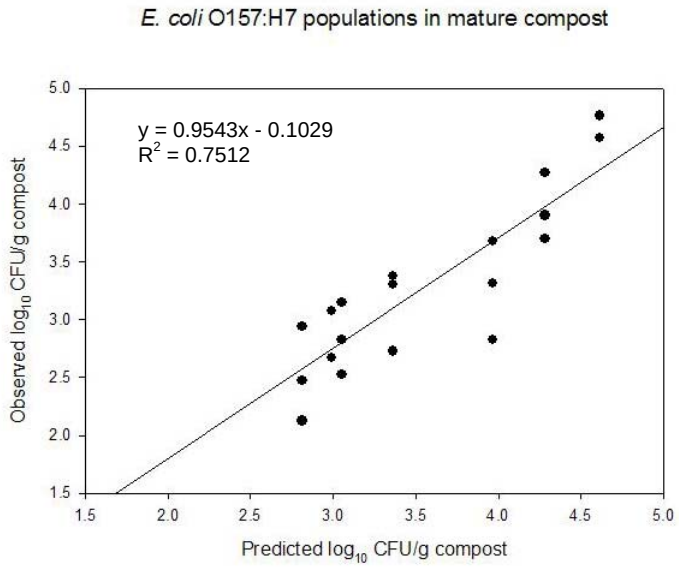

Figure 2. Comparison of predicted vs. observed *E. coli* O157:H7 populations in mature compost, as determined by enumeration of *E. coli* O157:H7 in compost and inocula on ntCHROMagar O157.

Related references:

Barkocy-Gallagher, G. A., K. K. Edwards, X. Nou, J. M. Bosilevac, T. M. Arthur, S. D. Shackelford, and M. Koohmaraie. 2005. Methods for recovering *Escherichia coli* O157:H7 from cattle fecal, hide, and carcass samples: sensitivity and improvements. J. Food Prot. 68:2264-2268.

Brichta-Harhay, D. M., T. M. Arthur, J. M. Bosilevac, M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2007. Enumeration of *Salmonella* and *Escherichia coli* O157:H7 in ground beef, cattle carcass, hide, and fecal samples using direct plating methods. In press.

Hu, U., Q. Zhang, and J. C. Meitzler. 1999. Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by multiplex PCR. J. Appl. Microbiol. 87:867-876.