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## **Most Probable Number Methods for Enumerating Salmonella and E. coli 0157H:7 in Environmental Waters**

Michael Jenkins, Dwight Fisher, Dinku Endale, Richard Lowrance, Robert Hubbard, Larry Newton, Paige Gay, and George Vellidis

### Abstract Text:

#### Background

Most agricultural animals such as beef and dairy cattle, swine, and poultry are a source of Salmonella and E. coli0157H:7. Watersheds with animal agriculture can adversely impact recreational waters and threaten public. To understand better and manage the fate and transport of these pathogens in agricultural watersheds a most probable number (MPN) method for each of them was developed to determine their concentrations in environmental waters.

#### Methods

Each MPN method begins with a filtration step. As much as 20 l of water is filtered through a FALP 293 mm membrane with a 1  $\mu$ m pore size. The filtered material is scrubbed off the filter with a brush in PBS, and consolidated by centrifugation. The pellet is resuspended in PBS and used to inoculate tubes of Tetrathionate for Salmonella and Laural Tryptose Broth (LTB) for E. coli0157:H7. The tubes are arranged in a 5-tube, four dilution scheme. Positive Tetrathionate tubes are inoculated onto Brilliant Green agar plates; presumptive Salmonella isolates are streaked for purity on Beef Heart Infusion (BHI) agar plates. Fresh colonies are stabbed into slants of Lysine Iron Agar and Triple Sugar Iron agar. If both slants are positive for Salmonella, confirmation is made with a TaqMan assay using primers and probe for the junction between the virulence genes SipB and SipC. Positive LTB tubes are spread on Sorbitol MacConkey agar plates for isolating colonies. Suspected E. coli0157:H7 colonies are inoculated into tubes of LTB-MUG and checked for gas production and non-fluorescence. Corresponding colonies are tested for glutamate decarboxylase (GAD) activity and latex agglutination. If positive for both GAD and latex agglutination, confirmation is made with a TaqMan assay using primers and probe for the virulence gene eaeA. MPN is calculated with FDA's preferred MPN method.

#### Results and Conclusions

These MPN methods have determined densities of Salmonella and E. coli0157:H7 as low as 0.1 MPN l-1 of surface water impacted by animal agriculture. They can be used as tools for better understanding fluxes of these pathogens in watersheds impacted by animal agriculture.

### Impact Statement:

These two MPN methods for Salmonella and E. coli 0157:H7 proved a set of quantitative tools for improving our understanding of the fluxes of these two pathogens in association with fecal indicator bacteria and total aquatic microbial community. This method has the capacity to develop collections of environmental isolates for purposes of microbial source tracking.