Laboratory Procedure **MFLP-87**
September 1996

HEALTH PROTECTION BRANCH

OTTAWA

DETECTION OF ENTEROHEMORRHAGIC *E. COLI* (EHEC) IN FOOD PRODUCTS AND FOOD INGREDIENTS BY THE VIP FOR EHEC METHOD

Don Warburton
Evaluation Division
Bureau of Microbial Hazards, Food Directorate
Postal Locator: 2204A1
Ottawa, Ontario, K1A OL2

1. APPLICATION

The method is applicable to the detection of enterohemorrhagic *E. coli* 0157 from food products and food ingredients to determine compliance with the requirements of Sections 4 and 7 of the Food and Drugs Act.

2. DESCRIPTION

VIP for EHEC is a visual immunoprecipitate assay that detects enterohemorrhagic *E. coli* including *E. coli* 0157:H7. It employs highly specific antibodies directed against EHEC antigens and has been specifically formulated to minimize cross-reactivity with many Enterobacteriaceae while maintaining superior sensitivity. The method has been shown to produce satisfactory results with contaminated foods in AOAC and HPB studies. This method can be used for the detection of EHEC in food products, food ingredients, and environmental samples.

3. PRINCIPLE

The VIP for EHEC method uses a proprietary reagent system to form an antigen-antibody- chromogen complex if EHEC is present. This ensures a high level of sensitivity and specificity to E. coli 0157:H7. Appropriately enriched samples are added to the VIP unit; any EHEC antigens will bind to the antibody-chromogen complex as it flows across a supporting membrane. When EHEC is present, the antigen-antibody-chromogen complex will form a detection line in the test sample window. Sample flow will continue down the membrane to form a control line in the test verification window regardless of whether the sample contains EHEC.

4. DEFINITION OF TERMS

See Appendix A of Volume 3.

5. COLLECTION OF SAMPLES

See Appendix B of Volume 3.

6. MATERIALS AND SPECIAL EQUIPMENT

1) VIP for EHEC (BioControl Systems Inc., phone: (206) 487-2055, (800) 245-0113, FAX: (206) 487-1476.

2) Modified Trypticase Soy Broth and Novobiocin (mTSB-n). See MFLP-80.

Published by: POLYSCIENCE PUBLICATIONS, P.O. Box 1606, Station St-Martin, Laval, Quebec, Canada H7V 3P9. TEL.: 1-800-840-5870. FAX: (450) 688-1930.

3) Colworth Stomacher 400, blender or equivalent.

4) Incubators capable of maintaining 35°- 37° C.

5) Water bath capable of maintaining 100 ± 2° (or flowing steam autoclave set at 100°).

6) Stomacher bags, with mesh inner bag (VWR Scientific)

7) Filter tips for pipettes (See MFLP-80).

7. PROCEDURE

Each sample unit may be analyzed individually or the analytical units may be combined. Carry out the test in accordance with the following instructions:

7.1 Handling of Sample Units:

7.1.1 In the laboratory prior to analysis, except for shelf-stable foods, keep sample units refrigerated (0-5° C) or frozen, depending on the nature of the product. Thaw frozen samples in a refrigerator, or under time and temperature conditions which prevent microbial growth or death.

7.1.2 Analyze sample units as soon as possible after their receipt in the laboratory.

7.2 Preparation for Analysis

7.2.1 Have ready sterile mTSB-n.

7.2.2 Clean the surface of the working area with a suitable disinfectant.

7.3 Preparation of Sample

7.3.1 To ensure a truly representative analytical unit, agitate liquids or free flowing materials until the contents are homogeneous. If the sample unit is a solid, obtain the analytical unit by taking a portion from several locations within the sample unit. To reduce the workload, the analytical units may be combined for analysis. It is recommended that a composite contain not more than 500 g.

Note: The use of stomacher bags with a mesh inner bag is needed with some samples (ex. spices).

7.3.2 Prepare a 1:10 dilution of the food by aseptically blending or stomaching 25 g or mL (the analytical unit) into 225 mL of the mTSB-n broth. Some spices have to be analyzed using a higher dilution (see MFLP 80).

7.3.3 Incubate overnight (at least 18 hrs) at 35°C.

7.4 VIP Assay Procedure

7.4.1 Open sealed pouch containing VIP units and remove required number of tests. One device is necessary for each test sample. VIP units may not be reused. Be certain to immediately reseal unused VIP units in pouch containing desiccant. Store at ambient temperature in a cool, dark location.

7.4.2 Gently shake enrichment broth, then allow food particles to settle.

7.4.3 Transfer 0.1 mL of inoculated broth to sample additional well.

Note: Sample particles may affect flow. It is recommended that filter tips be used when pipetting the sample.

7.4.4 Incubate at ambient temperature for 10 minutes.

7.5 Reading Results:

7.5.1 Examine VIP unit for the presence of a distinct line in the test verification window. This line should be dark in color when contrasted with the white background and extend across the window. Absence of a control line indicates an invalid test result. Contact BioControl Technical Services.

7.5.2 Observe test sample window. Presence of a distinct line, as described above, indicates a presumptive positive sample. Absence of a line is a negative. Differing intensities of test and control lines are acceptable as long as control line is present.

Note: Examine device at 10 minutes. Do not read results after 20 minutes, as faint lines may develop because of non-specific color development and should be disregarded.

7.5.3 Positive and negative control cultures should be run to familiarize the analyst with results interpretation.

7.5.4 Autoclave units at 121°C for 15 minutes prior to discarding.

7.6 Confirmation of Positive VIP Samples:

7.6.1 Presumptive positive samples must be confirmed culturally as described in MFLP 80. Prepare appropriate dilutions from mTSB-n. Spread plate to specified selective agars and confirm biochemically and serologically.