Australian Government
Department of Agriculture

BioControl VIP – AOAC 996.09

SCOPE

This method is suitable for the detection of *E. coli* O157:H7 in dairy products, meat, poultry, seafood and liquid eggs. The 8 h enrichment protocol using BioControl mEHEC (replacing EHEC8) media is suitable for testing raw and cooked beef.

PRINCIPLES

Specific antibodies for *E. coli* O157:H7 are bound to a chromogenic carrier and separately to a solid support. *E. coli* O157:H7 reacts with antibody-chromogenic complex and flows across a lateral flow membrane where it is bound by antibody immobilised on the membrane. Positive reaction is indicated by a coloured line in the sample test window. A line in the test verification window indicates that the analysis has proceeded correctly.

- **Enrichment**
  Sample is diluted in 9 x its weight of modified trypticase soya broth with added novobiocin (mTSB+N, FDA BAM[1]). Samples are then enriched overnight (18 – 28 h) at 35 – 37°C. The manufacturer recommends adding 2.25 ml of steamed (15 min) TritonX-100 to 225 ml of mTSB+N for viscous samples. For 8 h enrichment of raw and cooked beef samples. Prepare mEHEC media as per the manufacturers instructions, pre-warm to 42°C prior to use. Add the sample and mix, incubate for 8-12 h at 42°C. Both methods been validated for use with 375g samples enriched in one litre of broth.

- **Immunoassay**
  Carry out the VIP assay following the manufacturer's instructions. The device must be read immediately after 10 min incubation to avoid a false positive reading. Note for the 8h test enrichment broth is boiled prior to inoculation of the VIP device.

- **Confirmation**
  Isolation of *E. coli* O157 is carried out from the enrichment broth using an Immunomagnetic separation procedure ie ISO 16654:2001, FSIS MLG 5 or FDA BAM Chapter 4A. Confirmation must be carried out at a department approved laboratory.

[1] 30g trypticase soy broth; 1.5g bile salt No. 3 and 1.5g K2HPO4 (anhydrous) per litre H2O. After autoclaving add 0.2ml of 100mg/ml novobiocin.

CHECKLIST

Enrichment	**18 – 28 h Protocol**	
	Is mTSB+N warmed to 36 ± 1°C (for large quantities) prior to use?	
	Is novobiocin added on the day of use a rate of 20 mg per litre?	
	What weight of sample and volume of enrichment broth are used?	
	Is enrichment carried out at 35 – 37°C for 18-28 h?	
	8 h Protocol	
	Is mEHEC media used for the initial enrichment?	
	Is media pre-warmed to 42°C prior to use?	
	What weight of sample and volume of enrichment broth are used?	
	Is enrichment carried out at 42°C for 8-12 h?	
	Is the enrichment broth boiled prior to performing the assay (10 min)?	
	Is a positive control run with each batch of samples analysed?	
	Are reference cultures inoculated into primary enrichment broth at a level of 10 to 100 cells?	
Immunoassay	Are the manufacturer's instructions available and are they reproduced in the laboratory manual?	
	Are VIP units stored at room temperature in a cool dark place?	
	Are technicians familiar with positive and negative reactions?	
	Is incubation carried out at room temperature (10min for 18-28h and 15-20min for 8-12h)?	
Cultural confirmation	Is *E. coli* O157:H7 confirmed from mTSB+N at a department approved laboratory?	
	Is an approved method used for confirmation (ISO 16654:2001, MLG 5, FDA BAM Chapter 4A)?	
	Is IMS concentration included in the confirmation methodology?	