

Certification Report

AOAC Research Institute

Performance *Tested Method*
030202

PATHATRIX *E.coli* O157
(PE50)

manufactured by

Matrix MicroScience Ltd

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Fordham Road

Newmarket, Cambridgeshire CB8 7NY

United Kingdom



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The PATHATRIX *E.coli* O157 Test System has been validated and certified as a *Performance Tested Method™* by the AOAC Research Institute as an effective method for the detection and isolation of *E. coli* O157 from raw ground beef at low levels i.e. 1cfu/25g . The PATHATRIX system was show to have a 0% false positive rate.

The PATHATRIX *E.coli* O157 system represents a very significant improvement on the current USDA FSIS procedure with superior sensitivity for the detection of low levels of *E.coli* O157 in raw ground beef; and provides confirmed results in under 24 hours as opposed to 48 hours for the USDA FSIS method.

The PATHATRIX *E.coli* O157 Test System is a novel patented method that consists of a pre-programmed workstation and a consumable pack and employs magnetic beads coated with antibodies specific to *E. coli* O157. The raw ground beef sample is homogenised in a non-selective enrichment medium (Buffered Peptone Water) in a sterile stomacher bag containing a mesh liner. The bag is then placed on the PATHATRIX in a thermally controlled pot at 37°C and magnetic beads, coated with antibodies to *E. coli* O157, are added to the sample homogenate. The consumable pack is then loaded into the PATHATRIX system, and then the pre-programmed procedure is started. The liquid sample is then continuously re-circulated over the phase from the bag by a peristaltic pump via tubing (Figure 1). Within this closed loop system is a plastic phase, containing a metal grid, which is magnetised and captures the beads onto the grid as they pass.

After continuously circulating the sample around the system and through the phase for 180 minutes, the target organisms are bound to the magnetic beads onto the phase. Any residue and food debris is removed from the phase by a subsequent wash step. The beads from the capture phase are then eluted into a wash vessel and concentrated using a magnetic rack.

After completion of the capture step the sample can then be directly plated onto Sorbitol MacConkey agar supplemented with Cefixime and Potassium Tellurite (CT- SMAC). These plates are incubated at 37°C for 16-24h and examined for presumptive colonies of *E. coli* O157. Having used the system to isolate *E. coli* O157, the laboratory would then take a selection of typical colonies and subject these to confirmation of O157 antigen and biochemical identification of *E. coli*.

1.0 Target Organisms

Escherichia coli O157 (including H7)

2.0 Matrices

Raw ground beef

3.0 Summary of validated performance claims

Sensitivity	221% (by comparison to the USDA FSIS method)
Specificity	approx. 100 %

4.0 Accuracy/ Comparison to Existing Methods

PATHATRIX allows the detection and isolation of *E. coli* O157 from raw ground beef at low levels (1-10cfu/25g). The results of the comparative study are

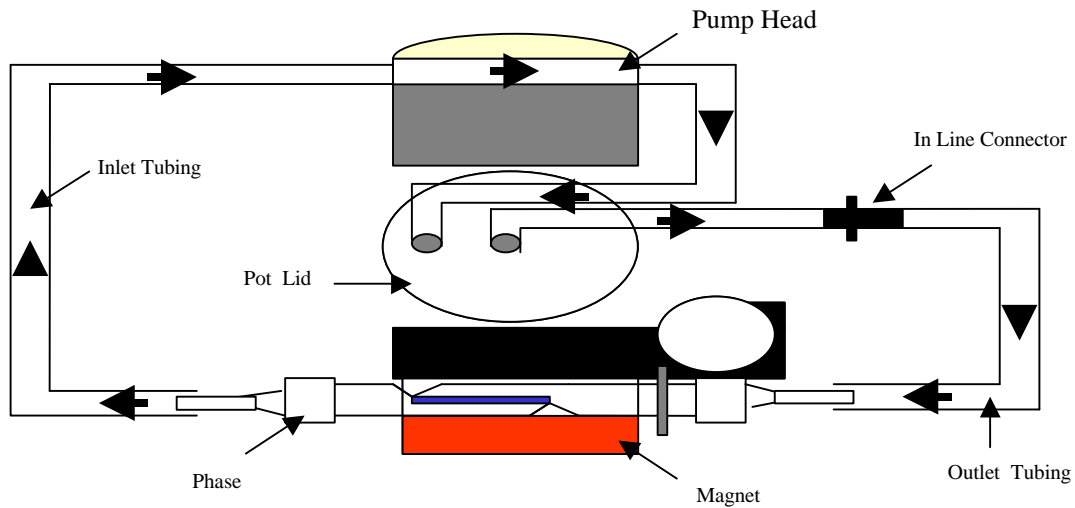
summarised in Table 1.

Table 1: Summary of Results Comparing the Detection of *E. coli* in Raw Ground Beef by the USDA FSIS Method and PATHATRIX *E. coli* O157 Test

Level of Contamination	PATHATRIX O157	USDA FSIS Culture Method
Low (5.25 cells/25g)	13/20	3/20
Intermediate (27.5 cells/25g)	20/20	10/20
Intermediate (23.5 cells/25g)	20/20	11/20
Total	53/60	24/60

The results showed that the PATHATRIX *E. coli* Test was superior to the USDA FSIS method for the detection of low levels of *E. coli* O157 in raw ground beef.

Figure 1 Schematic Layout of System : Plan View



5.0 TEST KIT INFORMATION

- 5.1 Kit name: PATHATRIX *E.coli* O157
- 5.2 Catalogue Number: PE50 (contains 50 sterile consumable packs and 50 test bead vial)
- 5.3 Ordering Information

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5.4 Test Kit Consumables and Reagents

- 5.4.1 Capture phase units - Pre-assembled, sterile, individually packed, capture phase units comprised of: a plastic moulded capture phase with moulded ends attached; a silicon tubing interconnecting the components; a pot lid; a foam bung; connector; heat seal pouch used as packaging.
- 5.4.2 Tube sets - Sterile, individually packed wash tube sets comprised of: a wash vessel with "spouted" lid and internal tube pre-assembled; a separate flat lid; a grip seal pouch used as packaging.
- 5.4.3 Vials containing 2.5ml (sufficient for 50 tests each of 50µl each) of *E. coli* O157 antibody coated magnetic beads.

6.0 ADDITIONAL SUPPLIES & REAGENTS

- 6.1 Buffered Peptone Water (Oxoid UK or equivalent)
- 6.2 CT-SMAC Sorbitol MacConkey Agar (Oxoid UK or equivalent)
- 6.3 Prolab latex agglutination system (Pro-lab Diagnostic or equivalent)
- 6.4 Biochemical tests (API 20E; bioMérieux or equivalent)

7.0 APPARATUS

- 7.1 PATHATRIX Workstation. The PATHATRIX workstation houses five independent units, each has its own incubation pot, heating element, motorised pump head and control panel.
- 7.2 Stomacher 400 (Sowards UK) with appropriate size of sterile bag with filter (Seward Cat No 6041/STR).
- 7.3 Incubator static $37\pm 1^{\circ}\text{C}$.
- 7.4 Micropipettes to deliver 15 -100 μl with sterile disposable tips.
- 7.5 Mechanical pipette with 1.0ml, 5.0ml, and 10.0ml with sterile disposable tips.
- 7.6 Sterile disposable inoculation loops, “hockey stick” spreaders.
- 7.7 Pipette bulb.
- 7.8 Top loading balance. – Sensitive to 0.1g, to weigh 25 g test samples