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## Isolation of Escherichia coli pathogens from oysters

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**Protocol status:** Working

**We use this protocol and it's working**

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- 1 The filtered homogenate was streaked on MacConkey's agar using standardized loops, the loops were flamed periodically to ensure sterility. This was done in duplicate. The plates were then inverted and incubated at 34°C. After an overnight incubation at 34°C, the plate with the best significant and adequate colonies was used to test for the presence of *E. coli*. Those colonies that were morphologically characteristic for *E. coli* were gram stained:
- 2 Each slide was labelled according to location and given a number. A circle using a wax pencil was drawn on the underside of each slide.
- 3 Drop of sterile saline solution was placed into the circle and using a slide on one side with a sterile forceps and passing the entire slide through the flame of a Bunsen burner two of three times with the smear side up. Making sure that overheating does not occur.
- 4 The heat fixed slide was placed on a straining tray.
- 5 The slide was gently flood with crystal violet and left to stand for 1 minute.
- 6 Using a wash bottle of distilled water, the slide was tipped slightly and gently rinsed.
- 7 Then using Gram's iodine, the slide was gently flood and let stand for 1 minute.
- 8 The slide was tipped slightly and gently rinsed with distilled water using a wash bottle.
- 9 95% ethyl alcohol was used to decolorize the smear by rinsing the slide for 5-10 seconds.
- 10 The slide was then immediately rinsed with water.
- 11 Using Safranin, the slide was gently flood to counter-stain and let stand for 45 seconds.
- 12 The slide was tipped slightly and gently rinsed with distilled water using a wash bottle.

- 13 The slide was then blot dried with bibulous paper.
- 14 Using a light microscope under oil immersion, the smear was observed to determine whether the colonies were gram negative or gram positive.
- 15 This was done per presumptive *E. coli* colony observed on each plate.
- 16 A single colony of presumptive *E. coli* was then subcultured on MacConkey's agar to purify the culture and incubated at 34°C. A single colony was later gram-stained prior to biochemical testing in order to ensure the culture's purity. The pure single colonies were further analysed via biochemical tests (Indole, Citrate, Urease, Motility and Triple Sugar Iron Agar) together with a control *E. coli* colony taken from the laboratory in order to make a comparison. A single colony of the organism was then saved in a cryovial containing Nutrient Agar at -70 °C. This procedure was done per presumptive *E. coli* spp found.
- 17 Reaction of the Non-Sorbitol fermenting *E. coli* on Sorbitol MacConkey agar.
- 18 The isolates were further confirmed as O157:H7 using the Hardy Diagnostics *E. coli* PRO™O157 Kit and the manufacturer's instructions were followed:
- 19 All reagents were kept to room temperature for at least 10 minutes.
- 20 One drop of sterile saline was placed within a circle on the test card.
- 21 1-4 well-isolated colonies were selected from the agar surface.
- 22 An emulsion was created by mixing of the colonies and the Saline on the test card.
- 23 The Latex Reagent was inverted and 1 drop of the *E. coli* PRO™O157. Latex Reagent was dispensed onto a test circle on the test card. The Latex Reagent bottle was not allowed to touch the organism suspension.
- 24 The Latex reagent and the organism suspension was mixed with the sterile wooden sticks provided, using the complete area of the circle. A new stick was used for each



reagent.

- 25 The entire card was gently rocked, allowing the mixture to flow slowly over the ring area.
- 26 For up to 2 minutes. under normal lighting conditions, agglutination of the latex particles was observed.
- 27 The method was repeated with the Negative Control Latex Reagent to rule out the change of a false positive result.