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Isolation of Shigella pathogens from oysters

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1 The filtered homogenate was streaked using standardized loops on Salmonella-Shigella (SS) agar, the loops were flamed periodically to ensure sterility. This was done in duplicate. The plates were then 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 Shigella. Those colonies that were morphologically characteristic for Shigella 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.

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3 Drop of sterile saline solution was placed into the circle and using a sterile loop, a single colony from the plate was taken and mixed into it.

4 The smear was allowed to air dry and then heat fixed by holding the slide on one side with a sterile forcep and passing the entire slide through the flame of a Bunsen burner two or three times with the smear side up. Making sure that overheating does not occur.

5 The heat fixed slide was placed on a straining tray.

6 The slide was gently flood with crystal violet and left to stand for 1 minute.

7 Using a wash bottle of distilled water, the slide was tipped slightly and gently rinsed.

8 Then using Gram's iodine, the slide was gently flood and let stand for 1 minute.

9 The slide was tipped slightly and gently rinsed with distilled was using a wash bottle.

10 95% ethyl alcohol was used to decolorize the smear by rinsing the slide for 5-10 seconds.

11 The slide was then immediately rinsed with water.

12 Using Safranin, the slide was gently flood to counterstain and let stand for 45 seconds.

13 The slide was tipped slightly and gently rinsed with distilled was using a wash bottle.

14 The slide was then blow dried with bibulous paper.

15 Using a light microscope under oil immersion, the smear was observed to the determine whether the colonies were gram negative or gram positive.

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16 This was done per presumptive Shigella colony observed on each plate.

17 A single colony of presumptive Shigella was then subcultured on SS agar and incubated at 34°C prior to biochemical testing. These pure single colonies were further analysed using biochemical tests (Indole, Citrate, Urease, Motility and Triple Sugar Iron Agar). The tests were done together with a control Shigella colony taken from the laboratory in order to make a comparison. A colony of the organism was then saved in a cryovial containing Nutrient Agar -70 °C. This was done per Shigella spp. found.

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