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Isolation of Salmonella pathogens from oysters

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- 1 The filtered homogenate was streaked using standardized loops on Salmonella Shigella agar (selective and differential media for Salmonella and Shigella); 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 Salmonella Those colonies that were morphologically characteristic for Salmonella are 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 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 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 andmgently 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 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 blot dry ice 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.
- 16 This was done per presumptive Salmonella colony observed on each plate.