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Appendix 1

Methods

Sample Handling

Primary samples were handled by using gloves. The sample collection bottles and tubes were removed from plastic bag and wiped with disinfectant wipes. After used, the bottles and tubes were wiped again with disinfectant wipes and placed in a lab rack.

Antibiotic Preparation

4 mg/L of cefotaxime antibiotic was made from stock 5 mg/L by using calculation:

Volume of agar that was made x 4 = volume of CTX from stock that had to be added x 5

Hicome Agar with and without CTX

Hicome agar was prepared by knowing the appropriate amount of powder to be added to certain amount of distilled water. From the label, it was written 27 grams of Hicome agar powder has to be added to make 1 L mixture. Then, the mixture was autoclaved and poured into appropriate plates.

For Hicome agar with CTX, appropriate amount of antibiotic was added by using given calculation before was processed to autoclave step.

MacConkey Agar

MacConkey agar was prepared by knowing the appropriate amount of powder to be added to certain amount of distilled water. From the label, it was written 51.5 grams of MacConkey agar powder has to be added to make 1 L mixture. Then, the mixture was autoclaved and poured into appropriate plates.

Mueller-Hinton Agar

Mueller-Hinton agar was prepared by knowing the appropriate amount of powder to be added to certain amount of distilled water. From the label, it was written 38 grams of Mueller-Hinton agar powder has to be added to make 1 L mixture. Then, the mixture was autoclaved and poured into appropriate plates.

Tryptic Soya Broth (TSB)

Tryptic soya broth (TSB) was prepared by knowing the appropriate amount of powder to be added to certain amount of distilled water. From the label, it was written 30 grams of TSB powder has to be added to make 1 L mixture. Then, the mixture was autoclaved and poured into appropriate plates.

.85% Saline

Point eighty five percent of saline was made by using combination of 8.5 gr of NaCl with 1 L of distilled water. The mixture was aliquot into universal bottle as much as 9 ml for serial dilution.

20% Glycerol Broth

One point zero four grams of Nutrient Broth powder was mixed with 80 ml of distilled water in 200 ml Duran bottle. Then, 20 ml of glycerol was added and mixed well. After that, the mixture was autoclaved to avoid any possible contaminations.