Appendix I

Culture media

1-Mannitol salt agar

formula of mannitol salt Agar media (PH7.3)

Lab-lemco powder ……1.0

Peptone……...10.0

Mannitol…….10.0

Sodium chloride…….75.0

Phenol red……0.025

Agar……..15.0

1.2 Preparation

Media was prepared according to instruction of manufacture as follow. 11.1 grams were suspended in 100ml distilled water. The preparation heated to boiling to dissolve the medium completely, then sterilized by autoclaving at 151bs pressure ($121^{O}C$) for 15minutes.After cooling to 50-55, and 20-25ml of molten preparation were poured into sterile disposable 90 mm in diameter petri dishes. Date the medium and give it a batch number (Collee *et al*., 1996).

2- Mueller-Hinton Agar

2.1 Formula of Mueller-Hinton Agar (PH7.4)

Beef infusion……..300.0g

Cas amino acids…….17.5 g

Starch……..1.5 g

Agar…….17.0g

Distilled water……1.000.0 ml

2.2 Preparation

Suspend 38.0 grams in 100ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 151bs pressure ($121^{O}C$) for minutes. Mix well before pouring.

Appendix II

Reagent and Stains

1. Gram's Stain

Most bacteria can be differentiated by their Gram reaction due to difference in the cell wall structure into Gram positive which after being stained dark purple with crystal violet are not decolorized by acetone or ethanol, and Gram negative which after being stained with crystal violet lose their colour when treated with acetone or ethanol, and stain red with safranine.

1.1 Required:

1- Crystal violet stains media.
2- Lugol's iodine HiMedia.
3- Acetone HiMedia.
4- Safranine HiMedia.

1.2 Method of preparation

1- The dried smear was fixed by heat or alcohol.
2- The fixed smear was covered with crystal violet for 30-60 minutes.
3- The stain was washed off with clean water.
4- Tip of all water, and the smear covered with lugol's iodine for 30-60 minutes.
5- The stain was washed off with clean water
6- Acetone was rapidly applied(Few seconds) for decolourization,and then washed rapidly with clean water
7- The smear then was covered with safranine stain for 2 minutes
8- The stain was washed off with clean water, and wipe the back of the slide clean.
9- After air-dry the smear was examined microscopically using immersion oil lens.

1.3 Result

Gram positive…….Dark purple

(Staphylococci appear as cluster forming (grape like) gram positive cocci).

Gram negative…….pale to dark red

2.Reagents

Hydrogen peroxide (3%$H_2O_{2)}$.

Undiluted human plasma (pooled).

Physiological saline.

1 M Hydrochloric acid.

3. Preparation of 0.5 McFarland standard

One percent v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to distilled water and mixed well. 1.175% w/v solution of barium chloride was also prepared by dissolving 1.175 gm. of Barium chloride in 100ml of D.W. the turbidity standard was prepared by adding 0.5 ml of 1.175%w/w barium chloride to 99.5 ml of 1% sluphuric acid solution, then mixed and transferred to a screw capped bottle, stored in dark at room temp (20-28^{o}c) and used for preparing the standard test and control inoculums. This standard has turbidity of suspension containing approximately 1.5x10^{8} bacterial cells/ml.

4. Antibiotic used in the study

Antibiotic disc used for double disc diffusion test (standardize test).

Antibiotic……Methicillin, Vancomycin,

Potency……..5µg.

Symbol…….M-5µg.

Source…… bioanalyse-Turky.

Appendix III

Table (4) showed Contents of kit (MuRSAflux)

Colour of label	Tube Labeling & contents	Number	No. of reactions
White	Buffer A	2 tubes	> 100 reactions
No colour	Oligo Master Mix	12 x 8 tubes	96 reactions
Blue	MRSA positive control Negative control	1 tube 1 tube	> 8 reactions > 8 reactions
No colour	RNase-free H_2O	1 tubes	> 100 reactions
Red	Primers : Forward mix (F) Reverse mix (R)	 1 tube 1 tube	 > 100 reactions > 100 reactions