SECTION- IV

REFERENCES
APPENDICES

1. Fresh venous blood
2. Heparinized syringes
3. RPMI 1640 (Hi media, Bombay)
4. Hydrochloric acid (Sisco Research Lab, Mumbai, India)
5. Sodium bicarbonate (Sisco Research Lab, Mumbai, India)
6. Penstrip or pencillin streptomycin, gentamycin, amphitericin (Antibiotics)
7. Phytohaemoagglutinin (Gibco USA)
8. Foetal Bovine Serum (Gibco USA)
9. Colchicine (Gibco USA)
10. Potassium chloride (Sisco Research Lab, Mumbai, India)
11. Acetic acid (Sisco Research Lab, Mumbai, India)
12. Giemsa powder (Sisco Research Lab, Mumbai, India)
13. Methanol (Sisco Research Lab, Mumbai, India)
14. Glycerol (Sisco Research Lab, Mumbai, India)
15. Sodium monohydrogen phosphate (Na$_2$HPO$_4$)
16. Potassium dihydrogen phosphate (KH$_2$PO$_4$)
17. Sodium chloride (Sisco Research Lab, Mumbai, India)
18. Trypsin (Sisco Research Lab, Mumbai, India)
PREPARATION OF REAGENTS

Culture media preparation:

16.8 gm of RPMI 1640 was dissolved in 500 ml of autoclaved double distilled water, then 2-3 gms of sodium bicarbonate was added to this solution, followed by the addition of 250 mg antibiotics namely penicillin (10,000 IU/ml), streptomycin (100ug/ml), gentamycin (2 ul/ml) and amphotericin. The volume was made to 950 ml by adding autoclaved double distilled water and the pH was adjusted to 7.7.1 and made up the final volume of the media to 1000 ml. The solution was filtered using positive pressure pump with 0.22–micron pore size cellulose filter (Millipore). The media was then aliquot into 15 ml vials and stored at -20°C.

Phytohaemoagglutinin (10%):

One gram of P form of phytohaemogglutinin was constituted with 10 ml sterile distilled water and stored in the refrigerator.

Colchicine (0.25%):

25 mg of Colchicine powder was dissolved in 100 ml of distilled water and stored in the refrigerator.

Hypotonic solution (0.075 M):

This was made by dissolving 560 mg of Potassium chloride in 100 ml of distilled water.

Physiological Saline (0.9%):

This was made by dissolving 900 mg of sodium chloride in 100 ml of distilled water.
Modified Carnoys fixative (3:1):

This fixative was made by mixing three parts of methanol and one part of acetic acid.

Stock Giemsa stain:

One gram of Giemsa was mixed with 20 ml of glycerol and stirred thoroughly for half an hour, once the granules were become fine powdered, remaining 30 ml of glycerol was added and kept in the incubator at 60°C overnight. Next day the mixture was brought to room temperature and filtration was done by adding 50 ml of methanol, then the filtrate was transferred to a brown bottle and stored in the fridge for any number of days. This was used as a stock solution.

Trypsin solution (0.4%):

20 mg of Trypsin was dissolved in 50 ml of physiological saline.

Phosphate buffer stock solution (A and B):

Solution A was prepared by dissolving 2.556 gms of disodium orthohydrogen phosphate in 500 ml of distilled water and solution B was prepared by dissolving 1.632 gms of potassium dihydrogen phosphate in 500 ml of distilled water.

Working Giemsa stain:

2 ml of Giemsa stain was mixed with 24 ml of phosphate buffer A and phosphate buffer B solutions.
Sterilization of the glasswares:

Glasswares and filtration apparatus were immersed overnight in a solution of Teepol (15 ml of Teepol (BDH) solution in 1 liter of water). Next morning it was brushed, cleaned, washed thoroughly in running water, rinsed in triple distilled water and boiled for an hour. Then it was dried in a hot air oven at 120°C, for one hour. After the glasswares were cooled to room temperature they were packed in brown paper and autoclaved at a pressure of 15 pounds per square inch for 20 minutes and cooled again to room temperature and stored in a sterile chamber.

Sample collection:

Five ml of venous blood was collected in a labeled heparinised syringe, using 21G needle. It was gently mixed with the heparin to prevent clotting and kept at room temperature until placed in a culture medium. Unacceptable specimens includes those that are clotted, unlabeled, mislabeled, drawn in the incorrect anticoagulant, more than 72 hrs old, contaminated, less than 1 ml in volume or exposed to extreme temperatures were not used to initiate the culture.


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