Lab. 6 human genetics

- **Roswell Park Memorial Institute medium (RPMI 1640)**: is a form of medium used in cell culture and tissue culture. It has traditionally been used for growth of human lymphoid cells. It is provided as liquid medium (Fig.1) or powder (Fig. 2).

Figure 1: Liquid RPMI 1640 culture medium.

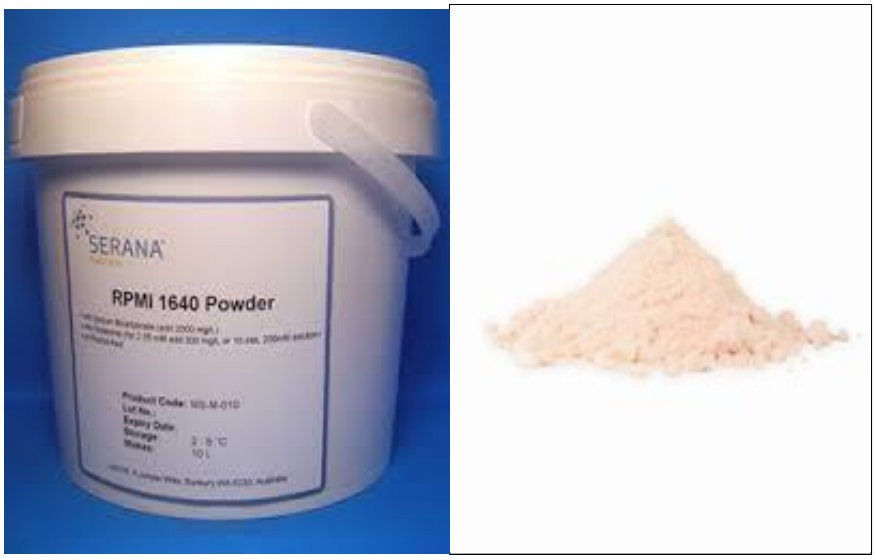

Figure 2: Powder of RPMI 1640 culture medium.

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Preparation of RPMI media:

- **Requirements:**

1. RPMI 1640 powder. is a widely used culture medium for a variety of cells, particularly for lymphocyte culture.
2. 10% (100ml) FCS (Fetal Calf Serum). The presence of Fetal Calf Serum (FCS) provides growth factors and hormones essential for lymphocyte activation and proliferation.
3. 1% PHA stimulates lymphocyte proliferation.
4. 2% sodium bicarbonate 10% (NaHCO3). helps maintain pH stability in the medium
5. 1% L-Glutamine (200mM). serves as a critical energy source for rapidly dividing cells.
6. 1% (10ml) antibiotic (penicillin10.000IU/streptomycin10.000µg\ml). helps prevent bacterial contamination during cell culture

- **Procedure : (To prepare 1 liter of RPMI 1640 medium):**

1. Add **700ml** distilled water to conical flask.
2. Measure powder media 15.8g.
3. Add **20ml** of 10% sodium bicarbonate (NaHCO3).Filter (NaHCO3) (with Millipore filter 0.45 µm pore size)
4. Add 1% **(10ml)** L-Glutamine (200mM)
5. Add 1% **(10ml)** antibiotic (penicillin10.000IU/streptomycin10.000µg/ml).
6. Add **160ml** distilled water.
7. Adjust the pH 7.2 - 7.4.
    1. **If pH is high:** add HCl (acid).
    2. **If pH is low:** add NaOH (base).
8. Filtering through Millipore filter with 0.22µm pore size.
9. Add 10% **(100ml)** FCS (Fetal Calf Serum). [**Note:**- Fetal Calf Serum must be warmed in water bath at 56˚C for 30 minutes to make inactivation of complement in FCS and eventually prevent agglutination of blood sample when added to media].

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Experiment (2): Culture of human peripheral blood lymphocytes.

Requirements:

3. Blood sample.
4. Lithium heparin tube (10ml).
5. Glass universal.
6. RPMI 1640 culture media.
7. Incubator.
8. Colcemid (0.1ug/ml). [It is used as the arresting agents. It cause blocking cell division at metaphase].
9. Universal Centrifuge.
10. KCl (0.075 M).
11. Acetic acid.
12. Methanol.
13. Microscope slide.
14. Microscope.

Procedure:

1. Blood is collected in a sterile 10 ml lithium heparin tube
   Inoculate about 0.8 ml of whole blood into a glass sterile universal container with 10ml media **(RPMI1640)**
2. Incubate the culture at 37C^{o} for 72 hr.
3. 2h prior harvesting add 0.1 ml of **colcemid solution** {final concentration 0.1ug/ml}
4. Transfer the culture to a centrifuge tube and spin at 500 g for 5 minutes.
5. Remove the supernatant and mix thoroughly and add 10ml of pre-warmed 0.075 M KCL **(hypotonic solution)** incubate at 37C^{o} for 10 minutes
6. Spin at 500g for 5 minutes
7. Remove the supernatant, mix thoroughly and add drop by drop 10 ml fresh **fixative** [made up of **1 volume acetic acid** to **3 volume methanol**].
8. Repeat steps 6 &7 twice more

9. spin at 500g for 5 minutes
10. Re suspend the cell pellet in a small volume of **fresh fixative** and drop on to a clean microscope slide from about **1 meter height** to pre-cooled slide and allow to dry.
11. Incubate the slides for 20-40 seconds in **trypsin solution** in coplin jar.
12. Rinse the slides thoroughly with cold Phosphate buffer saline (PBS) ph 7.0
13. Rinse the slides in distilled water and dry with air.
14. Stain for 5 minutes in Giemsa solution.