Blood Culture Technique for Human Chromosomes

The following account is based on the technique described by Moorhead et al., Exper. Cell Res. 20: 613,616, 1960.

Setting up cultures:

1. A 20 cc syringe is moistened with heparin solution (Sodium Liquaemin 50 mg/cc. Organon Inc., W. Orange, N.J.). Excess heparin is thoroughly expelled from syringe and 20 cc blood is aspirated under sterile conditions from a convenient vein. Put in 50 cc centrifuge tube.
2. Add 0.1 cc of Phytohemagglutinin M for each 5 cc of blood. Swirl tube to mix Phyto hemagglutinin with blood.
3. Leave in iced water 45 min.
4. Spin at 500 r.p.m. for 2 min.
5. Aspirate supernatant fluid (containing white cells) 2 - 3 cc usually, and transfer to a clean 1 oz. French culture bottle containing 6 cc of #199 media.
6. Do a W.B.C. on supernatant. This is not essential but will indicate whether a satisfactory yield is being achieved.

   Haemocytometer count of nucleated cells.

   a. Pull up to 1.0 m on counting pipette and dilute with acetic acid to 101 = 1:10 dilution.
   b. Each large square on slide (9/side) = 10^{-4} ml.

      Therefore, number of cells counted in 1 large square are counted by a factor 10^5 = cells/ml original solution.
   c. Blood cells should be 1-3 X 10^6 / ml, or 10-30 cells per large square.
7. Leave cultures in water bath at 37° C for 3 days.

Preparation of Chromosomes from culture:

1. Approximately 4 hrs. before harvesting add 0.9 cc of 0.0004% colchicine to ea. bottle.
2. After 4 hrs. treatment with colchicine, swirl bottle & transfer contents to a 12-15 cc centrifuge tube. Centrifuge at 800 r.p.m. for 5 min.
3. Remove supernatant.

4. Add 5 cc of Balanced Hank's solution (warmed at 37°C). Mix and resuspend cells. Centrifuge at 8 r.p.m. for 5 min.
5. Remove supernatant except for 1/2 cc of the Hank's solution.
6. Add 1.5 cc of warm distilled water. Resuspend cells.
7. Incubate 4 min. in 37°C water bath.
8. Spin 4 min. at 600 r.p.m.
9. Quickly remove supernatant.
10. Add freshly prepared fixative (Glacial Acetic : Methanal 1:3) without disturbing cell button. Cork tubes and refrigerate for 1 hr.
11. Resuspend cells. Centrifuge at 602 r.p.m. for 5 min. Remove supernatant. Add fresh fixative mixing up cells.

Slide Preparation:

Slides should be cleaned in 70% alcohol containing 1% IN HCl.

1. Dip slide in cold water in beaker. Upon removal, an even layer of water should cover slide. Drop 5-6 drops of cell suspension onto slide. Absorb excess water from slide edges with absorbent paper.
2. Shake slide vigorously until dry.

Staining:

1. 1% "Michrome" Orcein (natural) in 45% acetic acid.
2. 95%
3. 95%
4. Absolute
5. Absolute
6. Xylene
7. Xylene
8. Cover slip using permount or Canada balsom.

Media and Equipment

1. 50 cc centrifuge tubes
2. 12 or 15 cc centrifuge tubes
3. Corks to fit bath
4. Aspirating pipettes
5. 1 oz French bottles (culture) - Harshaw
6. Sodium Liquaemin 6 X 10 cc vials - Organon Inc., W. Orange, New Jersey
7. Bacto Phytohemagglutinin M - Difco
8. Mixture #199 with Bicarbonate - Microbiological Associates (Bethesda, Md.)
9. Antibiotics - mixture of Penicillin and Streptomycin used - 100 units/cc of media.
   To prepare add 1,000,000 units crystalline Penicillin (Potassium-Pfiger) and 1,000,000 units Streptomycin Sulfate (2 cc - Lilly) to 50 cc H_2O in sterile graduate. Fill up to 100 cc with sterile H_2O. Mix in separate flask and dispense in screw top tubes, approx. 5 cc/ tube. 10,000/cc.
   Add 1 cc per 100 cc of media.
10. Orcein ("Michrome" - natural) (Edward Gurr, Ltd., 42 Upper Richmond Road West, London, S.W., 14, England)
    in 45% acetic. Reflux for 2 hrs., allow to cool and filter. Refilter before use with Whatman No. 50 filter paper.
11. Colchicine - 1 gm. amounts - Nutritional Biochemical Corp., Cleveland, Ohio.