

### Method for Velocity Sedimentation Ig Gradient

Prepare 2500 ml MEM - Earle's Balanced Salt Solution. (Can do it with 2.25L)

Catalogue No. K-11, GIBCO.

Use 9.69 grams/litre MEM (21.8gms/2.25liters)

Use 2.2 grams/litre sodium bicarbonate (4.95gms/2.25liters)

Prepare 200 mls MEM (3% FCS – inact. 56°30')

6mls FCS → 200mls

60 mls MEM (5% FCS)

3mls FCS → 60mls

940 mls MEM (15% FCS)

141mls FCS → 940mls

940 mls MEM (30% FCS)

282mls FCS → 940mls

Wash equipment with hot, cold and distilled water. No soap.

Dry with paper towels.

Stand in hood for 1hr. with germicidal light. Replace gradient maker in cold room.

Join all tubing except at A. Use syringe filled with MEM (0) to fill tubing going down into chamber.  
Turn off B tap.

Connect A when tubing is full. Put enough MEM (0) in chamber I to cover metal.

Proceed to fill tubing between gradient chambers with MEM (0) so that all tubing is full, with no air bubbles.

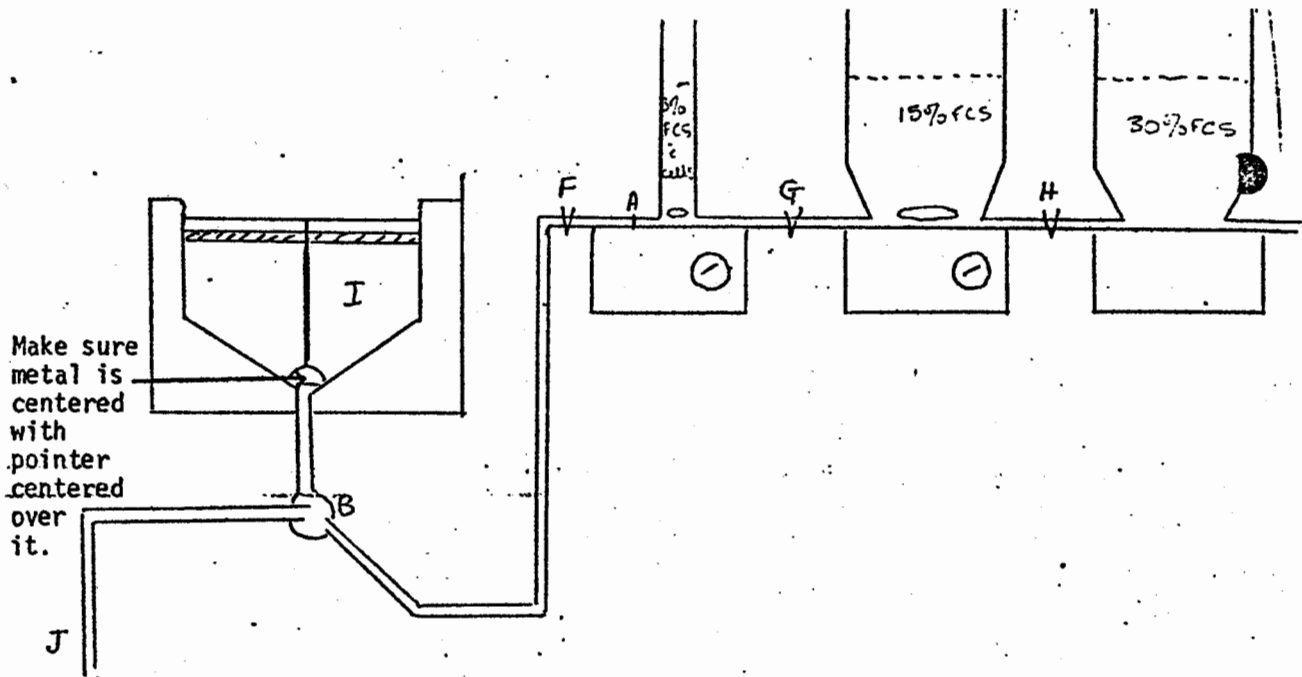
Empty chambers C, D, & E of excess buffer.

Put clamps at F, G & H.

Fill chamber E with the 940 mls of 30% MEM (FCS).

Fill chamber D with the 940 mls of 15% MEM (FCS).

Gradient is now ready for the cells.



### When Preparing Cells

Tease spleen through very small mesh grid with syringe plunger.

Spin 1500 for 10' in MEM (3).

Wash in MEM (3%)

Resuspend in ~20mls

Push slowly through 25g X 2 needle.

Push slowly through 27g X 2 needle.

(Push through little piece of cotton wool in syringe to remove clumps) pub not use TDC

Put cells ( $2 \times 10^8$ ) in 80 mls 3% MEM (more in our case)

This is loaded onto column.

For TDC treat in same way from \* down.