BLOOD DILUTING FLUIDS

Dilution Method for RBC and WBC Counting:

Fluids used as diluents must be isotonic, and have a high specific gravity which prevents the cells from setting too quikly.

Perpare dilutions for red cell counting by taking 0.02 ml of blood and washing it into 4ml of diluting fluid (R1) contained in a suitable container (this gives a 1 in 200 dilution).

White cells can be similarly diluted by taking 0.05 ml of blood into 0.95 ml of white cell diluting fluid (R2) (this gives a 1 in 200 dilution). Mixing is facilitated in these procedurse by the large bubble of air in the container, which is much more satisfactory.

Platelet Count:

Blood is diluted 1 in 20 by taking 0.1 ml of blood into 1.9 ml of diluent (R3). The diluted blood is well mixed and a Neubauer counting chamber filled with the diluted blood, after blowing out at least a third of the contents of the bulb. The Chamber is then stood in a petri dish, containing a piece of moist filter paper, for 20 min to allow the cells to settle. The moist filter paper prevents evaporation during the long period of standing. The cells are counted as for red cells (that is in 80 small squares). Some workers prefer to use phase contrast microscopy for counting platelets.

Erythrocyte Sedimentation Rate (ESR):

The ESR is of great value to the clinician and is commonly used as a screening test at the initial examination of the the patients. An increased rats of fall in chronic conditions such as rheumatoid arthritis and tuberculosis is usual , and the increase or decrease in ESR is used to monitor the progress of the disease. It is also elevated in acute and chronic infections and the malignant diseases where the plasma proteins are abnormal.
A decreased rate of fall is often present in polycythaemic subjects and a reading of 0 mm is not unusual. Certain physical conditions also affect the rate of sedimentation and stringent precautions are therefore necessary to bring about standard conditions for the test.
There are two main methods of performing the ESR, namely Wintrobe's and Westergren's method.

Westergren Method:

1. **Westergren ESR tube. This tube looks rather like a 1ml pipette. It is 300 mm long with an internal diameter of 2.5 mm. It is graduated from the bottom over a 200 mm scale in millimeter divisions.**
2. **Venous blood taken into sodium citrate (R4). One Part of sodium citrate (R4) to 4 parts of blood.**
   The blood is well mixed and sucked into the tube to the top mark (0 mark).The Tube is then stood vertically for 1h.
   The level of the red cells is then read as the ESR. Normal values are: men, 3-5 mm, and women, 4-7 mm.

Precautions to be taken when performing ESR tests:

1. As blood is taken by venepuncture prolonged venous congestion must be avoided.
2. The test must be set up within 3h of blood collection or sedimentation will be retarded.
3. Haemolysed blood must not be used.
4. Blood containing the slightest trace of a clot must be discarded.
5. Test must not be performed in direct sunlight.
6. Test should be performed between 18˚C and 22˚C as higher temperatures accelerate the sedimentation rate.
7. All the apparatus used must be clean.
8. There must be no air-bubbles in the sedimentation tube.
9. The tube must be placed in an absolutely vertical position.

BIODIAGNOSTIC
DIAGNOSTIC AND RESEARCH REAGENTS

BLOOD DILUTING FLUIDS	
Store at	15 - 25 ºC
In vitro diagnostic use	
CAT. NO.	DF 27 26

REAGENTS

R1	RBCs	100 ml	(25 T)
R2	WBCs	100 ml	(100 T)
R3	Platelet	100 ml	(50 T)
R4	ESR	100 ml	(350 T)

CONTACTS

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