R.B.C. Diluting Fluid (Hayem's)

It is used as diluting fluid for blood specimens to count red blood cells under high powder.

**Composition**

**Ingredients**
- Mercuric chloride: 0.25 gm
- Sodium sulphate: 2.50 gm
- Sodium chloride: 0.50 gm
- Distilled water: 100.0 ml
- Final pH (at 25°C): 5.9±0.1

**Formula adjusted, standardized to suit performance parameters**

**Directions**
1. Draw EDTA anticoagulated blood to exactly the 0.5 mark of the RBC pipette.
2. Wipe the tip of the pipette, clean with a piece of dry gauge without touching the opening of the capillary and immerse in the freshly filtered diluting fluid.
3. Do not insert the pipette in the bottle of counting solution.
4. By gentle mouth suction, draw the diluting fluid steadily into the pipette to exactly the 101 mark past the bulb, rotating the pipette on its long axis to ensure thorough mixing of blood and diluent.
5. Immediately mix the contents of the pipette thoroughly by placing the thumb over one end and shake for 1 minute.
6. Diluted blood must be examined within 2 hours.

**Principle And Interpretation**

RBC diluting fluid is isotonic with blood, hence hemolysis does not take place. Normal Saline also can be used. But it causes slight creation of red blood cells and allows rouleaux formation.

The blood specimen is diluted 1:200 with the RBC diluting fluid and cells are counted under high power (40 x objective) by using a counting chamber.

The number of cells in undiluted blood are calculated and reported as the number of Red cells per cu mm (MI) of whole blood.

**Quality Control**

**Appearance**
- Colourless, solution.

**Clarity**
- Clear with no insoluble particles.

**Reaction**
- Reaction of the solution at 25°C
- pH: 5.80-6.00

**Results**
- Under high power magnification, count the cells in the centre and in the four corner squares of the central ruled area.

**Calculation**

Red blood cells/mm³ in the original blood = \( \text{Cell counted} \times \text{dilution factor/Volume counted in mm}^3 \)  
\[ \text{Calculation} \]
= \( \text{Cell counted} \times 200 /0.02 \text{mm}^3 \)  
= \( \text{Cell counted} \times 10^4 \)
Storage and Shelf Life
Store below 30°C in tightly closed container and away from bright light. Use before expiry date on label.

Reference
1) Text book of Medical Laboratory Technology: Praful B.Godkar