**W.B.C. Diluting Fluid**

**Intended Use**
WBC diluting fluid is used for performing the WBC (Leucocyte) count.

**Composition**

**Ingredients**
- Glacial acetic acid: 2.00 ml
- Gentian Violet (1% w/v): 1.00 gm
- Distilled water: 97.00 ml
- Final pH (at 25°C): 2.2±0.2

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

**Directions**
1) Draw EDTA anticoagulated blood to 0.5 mark in the capillary end of WBC pipette.
2) Carefully, wipe excess blood outside the pipette by using cotton.
3) Draw diluting fluid up to 11 mark.
4) Mix the contents in pipette and after 5 minutes by discarding few drops, fill the counting chamber and allow the cells to settle for 2-3 minutes.
5) Focus on 1 of the "W" marked areas (each having 16 small squares) by turning objective to low power. 10X
6) Count cells in all 4 "W" marked corner squares.

**Principle And Interpretation**
WBC diluting fluid is used for performing the WBC (Leucocyte) count. Glacial acetic acid lyses the red cells. Gentian violet slightly stains the nuclei of the leucocytes. The blood specimen is diluted 1:20 in a WBC pipette with the diluting fluid and the cells are counted under low power of the microscope by using a counting chamber. The number of cells in undiluted blood is reported per cumm (µl) of whole blood.

**Warning and Precautions**
In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

**Performance and Evaluation**
Performance of the product is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

**Quality Control**

**Appearance**
Purple coloured, clear solution.

**Clarity**
Clear with no insoluble particles.

**pH**
2.00-2.40

**Calculation**
Number of WBCs/cumm(µl) of whole blood = No. of WBCs counted X Dilution /Area counted X Depth of fluid where, Dilution=20; Area counted= 4x1sq.mm=4sq.mm ; Depth =0.1mm(constant)

No. of leucocytes / cu mm(µl) of whole blood = No. of cells counted X 20 / 4 X 0.1 = No. of cells counted X 50

Please refer disclaimer Overleaf.
Storage and Shelf Life

Store between 10-30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Text book of Medical Laboratory Technology; Praful B. Godkar