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**NAGEOTTE COUNTING CHAMBER SUGGESTED METHOD FOR
COUNTING LEUKOCYTES IN RED CELL PRODUCTS**

1.0 SCOPE

- 1.1 This counting method describes a procedure for visual counting of leukocytes present in leukodepleted blood or packed red cells products.
- 1.2 The sensitivity of this method is 0.1 leukocytes/ μ L and should be used for procedures in which leukodepletion has reduced the count to levels below 5 leukocytes/ μ L as determined in the Hausser counting chamber.

2.0 EQUIPMENT

- Nageotte counting chamber with large size cover slips (supplied with chamber)
- Turk's Solution
- 10 - 100 μ L pipettor
- 100 - 1000 μ L pipettor
- 10 - 1000 μ L pipette tips
- 12 x 75 mm plastic test tubes
- Laboratory microscope (10x eyepiece) equipped with a 20x objective
- Covered petri dish containing moist filter paper

3.0 PROCEDURE

- 3.1 Label test tubes.
- 3.2 Add 900 μ L of Turk's Solution to each tube.
- 3.3 Make a 1:10 dilution of each sample by adding 100 μ L of each sample to the appropriately labeled test tube. Flush pipette tip several times to insure transfer of sample into diluent.
- 3.4 Mix diluted sample well by vortexing and allow to sit for 10 minutes (not longer than 1 hour) to lyse red cells.
- 3.5 Mix diluted sample again and withdraw 600 μ L into a pipettor. Carefully place the cover slip on a clean, dry Nageotte chamber. The cover slip must be centered exactly on the chamber. Carefully load the Nageotte counting chamber, without disturbing the cover slip, until it is full. Load from one side of the chamber only to prevent air from being trapped. Do not disturb the cover slip once the chamber is loaded.
- 3.6 Allow the chamber to rest in a moistened petri dish for 15 minutes to allow leukocytes to settle. Count the sample within 30 minutes.
- 3.7 Count leukocytes by scanning back and forth across the gridded area. Count all leukocytes in the area (i.e. 40 rectangles) including the cells touching all of the lines.
- 3.8 Count all 40 rectangles in one gridded area – this is the first count.
- 3.9 Move to second gridded area (either above or below the one already counted) and count the similar area as in 3.7-3.8. This is the duplicate count.
- 3.10 To calculate leukocytes/ μ L use the following formula:

$$\text{leukocytes}/\mu\text{L} = \frac{\text{cells counted} \times \text{dilution}}{\text{volume counted } (\mu\text{L})}$$

If one gridded area is counted the volume is 50 μ L. If both grids are counted the volume is 100 μ L.