**MANUAL RETICULOCYTE COUNT PROCEDURE**

A. **PRINCIPLE:** The reticulocyte is a non-nucleated immature red cell containing residual RNA. A supravital stain, new methylene blue, is used to precipitate the RNA into dark-blue filaments or granules to identify retics.

B. **SPECIMEN and REAGENTS:** EDTA whole blood is the preferred anticoagulant; New Methylene Blue Staining solution, 12x75mm tubes, pipets, glass slides.

C. **PROCEDURE:**
   1. Put 2 drops of new methylene blue in the bottom of a 12x75mm tube. Using a pipette, add 2 drops of well-mixed EDTA blood to the tube.
   2. Mix blood/stain mixture. The mixture color should be smoky-gray. Adjust if needed, i.e., add more blood if mixture is too blue.
   3. Incubate mixture at least 5 minutes but no longer than 10 minutes.
   4. **MIX** solution again….important! Prepare 2-4 good smears, **LABEL** and let dry.
   5. **Counting:** Using oil/100x power, count 500 total red cells separating mature RBC's from retics (use two counter keys). Retics are greenish with blue precipitates of RNA. **Two “dots” or more is a retic.** Go from feather edge to body of smear, making sure you are not counting too thick.
   6. Two techs count 500 RBC's on different retic smears for a total of 1000 RBC's counted.
   7. **Quality control:** The number of retics/500 RBC’s must agree + 2 retics between techs to accept results or another slide is counted. Controls must read within the assayed range to accept results.
   8. Both a relative percent retic and an absolute retic are reported:
      a. **Relative number** - # of retics in total of 1000 RBC’s = **percent (%)**.
      b. **Absolute number** - retic % x the RBC count/cmm = **thousands/cmm**.

D. **CALCULATIONS:**
   1. The relative reticulocyte count uses the sum of the two techs answers and the percent is reported to the nearest tenth (one decimal):
      \[
      \frac{\text{# retics in 1000 RBCs}}{10} = \% \quad \text{OR} \quad \frac{\text{# retics}}{1000 \text{ RBCs}} = \%.
      \]
   2. The absolute reticulocyte count is reported to the nearest thousand/cmm using the following calculation:
      \[
      \frac{\text{# retics/cmm}}{1000 \text{ RBCs}} = \frac{\text{# retics}}{1000 \text{ RBCs}} \times \frac{\text{RBC millions/cmm}}{\text{OR}} \frac{\text{retic %}}{100} \times \frac{\text{RBC/cmm}}{100}
      \]

E. **SOURCES OF ERROR:**
   1. Inadequate mixing before making smears
   2. Counting artifact or other inclusions as retics……black/shiny inclusions are “junk”.
   3. Improper ratio of blood to stain.
   4. Not counting all of the retics…..two blue “dots” or more is a retic.
   5. Wrong calculation