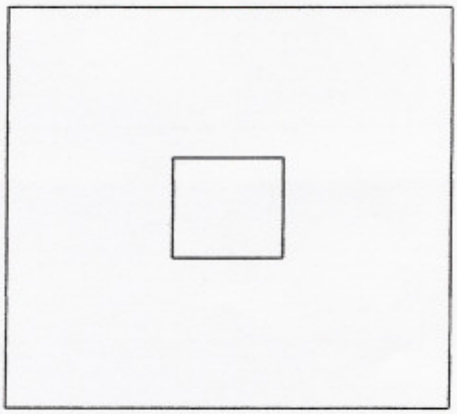
KR-802



KR-802A

MILLER SQUARES - Total ruled area: 7mm x 7mm
For counting components of greatly varying densities in a specimen. The frequency of the sparsely distributed particles in the major square is related in the proportion 1:10 to that of the denser populations counted only in the smaller square.

A squared reticle can be used for counting. Here the basic principle is that a small area of the specimen is analyzed in order to obtain information about the total area. This removes much irksome work and is preferable in certain instance to analyzing the whole area. For example when it is desired to compare the proportion of large to small particles in a specimen, by using the Miller Disc, the smaller particles may be counted only in the small square, the result being multiplied by ten for comparison with the number of larger particles in the large square.

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KR-802 & KR-802A ..... Miller Squares

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RETICULOCYTE COUNT

PRINCIPLE: When young red cells are stained with brilliant cresyl blue or other vital stains that enter the cell before fixation, the ribonucleoprotein is precipitated, appearing as a blue network or reticulum in the cell which is then called a reticulocyte.

REGANTS: Brilliant cresyl blue, prepared a follows:

1. 100 ml. Of 0.85% NaC1
2. 0.4 gm, sodium citrate
3. 1.0 gm. Brilliant cresyl blue
4. Mix well and filter before use. Store in brown bottle.
5. Mark 1 year expiration on label. Product is stable as long as slides stain properly.

SPECIMEN: Purple top tube or microtainer with EDTA as the anticoagulant.

PROCEDURE:

1. Label a small plastic capped vial with the patient identification and the time at which the smears can be prepared: (The time should be approximately 15 minutes after preparation.)

2. Using an MLA pipettor (50 ul or 100 ul), pipette equal volumes of the stain and blood into the vial. Mix well.

3. After about 15 minutes to allow for the cells to take up the stain, prepare 2 smears and dry quickly to prevent precipitation of the stain. Label smears.

   NOTE: While counterstaining is not recommended, there may be situations in which it is necessary so that an accurate result can be reported, e.g., the presence of high number of Heinz bodies. In such a situation, conterstain by staining the smear with Wright's stain procedure just as one would stain a peripheral smear.

4. There are two acceptable methods for manually counting retics:

METHOD A:

1. Using a 100x oil immersion objective and a 10x ocular, count the reticulocytes and RBCs on the smear. Randomly select areas for counting within the part of the smear where cells are close to each other but not touching or overlapping on a wedge smear.

2. Count a total of 1,000 red cells from a suitable number of fields.

METHOD B:

1. Using a 100x objective and a 10x ocular fitted with a Miller disc or suitable substitute, count the reticulocytes and RBCs on the smear. Randomly select areas for counting within the part of the smear where cells are close to each other but not touching overlapping.

2. COUNT A TOTAL OF 20 FIELDS. In each field, count RBCs in the smaller square and reticulocytes in the larger square. The proportion of reticulocytes (p) is computed as:

$$P = \frac{\text{total retics in larger squres}}{\text{total RBCs in smaller square x 9}}$$

5. Save one of the smears in case there is any question about the results over the next few days. However, please keep in mind that sometimes the retics will fade with standing, making them more difficult to see.

NORMALS: 0.5 - 1.5% for an adult.
2.5 - 6.5% at birth, falling to normal adult levels by the end of the second Week.

SUMMARY: Reticulocytopenia (decreased retic count: is seen in aplastic anemia and pernicious anemia. Reticulocytosis (elevated retic count) can be seen inhereditary spherocytosis, sickle cell anemia, PNH, thalassemia major, acquired autoimmune hemolytic anemia, erythroblastosis fetalis and acute posthemorrhagic anemia. There will also be increased retic counts seen in a favorable response in the treatment of some disorders such as pernicious anemia and iron deficiency anemia.

REFERENCE: Miale, Laboratory Medicine, Hematology, 3rd ed., p. 1125
Platt, Color Atlas and Textbook of Hematology, p. 182.

NCCLS Publication, Method for Reticulocyte Counting, H16-P
Vol. 5, No. 10, pp. 227-228

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