For in vitro diagnostic use.
For use in the detection of immature erythrocytes.

Specimen Collection
Fresh whole blood from EDTA anticoagulated blood and bone marrow specimens. It is not recommended to use blood over 24 hours old. If blood is refrigerated at 2-8°C, allow specimens to warm to room temperature.

Mode of Action
In 1949, Brecher developed a stain, new methylene blue, to identify reticulocytes based on aggregations of ribonucleic acid (RNA) within disintegrating ribosomes. The amount of RNA is the greatest when the erythroblast has just discarded its nucleus and decreases until its complete absence from the mature erythrocyte. Reticulocytes are immature erythrocytes and contain two or more blue stained granules. The quantitative reticulocyte count, along with other hematological parameters, is used in the evaluation of erythropoiesis.

Technical Procedure

Staining Protocol
1. Combine 3 drops of completely mixed blood with 2 drops of Reticulocyte Stain in a glass test tube and mix well.
2. Allow mixture to stand for 15 minutes at room temperature or incubate mixture at 37°C for 15 minutes.
3. Prepare wedge smears using the blood/stain mixture on glass slide.
4. Air dry smears for 15 minutes.
5. Coverslip and examine smears under a microscope.

Results
Red Blood Cells – Light to Medium Green
Reticulocyte Granules – Blue

Quantitative Study
Find area in smear using 10x (low power objective) where smear is thin and uniform and RBC’s are evenly distributed. Change to 100x (oil immersion objective) where there are at least 1000 RBC’s in the field.

Reticulocyte Count (%) = Total # of Reticulocytes

Normal adult reticulocyte counts = 0.5-1.5%

Discussion
Thermo Scientific™ Richard-Allan Scientific™ Reticulocyte Stain Solution should be stored at room temperature. This reagent is for “In Vitro” use only. Refer to the Safety Data Sheet for Health and Safety Information. This reagent is stable and should not form precipitants under ordinary storage parameters. If a precipitate does form, filter the stain through conventional lab grade filter paper. All dyes used in this formulation have been certified by the Biological Stain Commission.

Technical Comments
A counterstain is not recommended for this procedure. This procedure may require modification to suit personal preference.

References

Order Information

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