Brilliant Cresyl Blue Solution

Intended Use:
Brilliant cresyl blue solution is used in hematology as staining solution to examine reticulocytes in blood film.

Composition**

Ingredients
- Brilliant cresyl blue: 1.00 gm
- Sodium chloride: 0.85 gm
- Distilled water: 100.00 ml

**Formula adjusted, standardized to suit performance parameters

Directions
1) Add 2-3 drops of Brilliant cresyl blue solution in to 75-X10-mm plastic tube by plastic Pasteur pipette.
2) Add 2-4 volume of patient's EDTA-anticogulated blood to Brilliant cresyl blue solution and mix it properly.
3) Keep the mixture at 37°C for 15-20 min.
4) Resuspend the red cells by gentle mixing, and make films on glass slides in usual way.
5) Allow to air dry, and the films are examined without being fixed or counterstained.

Principle And Interpretation
Reticulocytes are juvenile red cells, which contain remnants of the ribosomal ribonucleic acid (RNA) that was present in larger amounts in the cytoplasm of the nucleated precursors from which they were derived. Ribosomes have the property of reacting with certain basic dyes such as of Brilliant cresyl blue to form a blue or purple precipitate of granules or filaments. This reaction takes place only in vitally stained unfixed preparations. Stages of maturation can be identified by their morphological features. The most immature reticulocytes have the largest amount of precipitable material. In the least immature only a few dots or short strands are seen. The regenerative capacity of erythrocytes can be monitored with ratio of reticulocytes.

Type of specimen
Clinical samples: Blood sample

Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines(1, 2).
After use, contaminated materials must be sterilized by autoclaving before discarding.

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.

Limitations
1) If blood films is allow to dry and is afterwards fixed with methanol, reticulocytes appear as polychromatic red cells staining diffusely basophilic if the film is stained with one of the basic dyes.
2) Staining ability of Brilliant cresyl blue varies from sample to sample.
3) A larger proportion of anaemic blood and smaller proportion of polycythaemic blood should be added in Brilliant cresyl blue solution than of normal blood.
4) Count of reticulocytes will tend to decrease after 6-8 hours unless the blood is kept at 4°C.

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

Please refer disclaimer Overleaf.
**Quality Control**

**Appearance**
Intense blue solution.

**Clarity**
Clear without any particles.

**Results**
When stained with brilliant cresyl blue the reticulocytes stain individually and display dark blue network and dark blue dots. The reticulocyte count is expressed in relation to 1000 counted erythrocytes (i.e. as °/00).

**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.

**Reference**
5. Dacie and Lewis Practical Haematology;Tenth edition;S.M.Lewis,B.J.Bain,I.Bates.