AG- GIEMSA STAIN (IFU)

PRINCIPLE:

AG- Giemsa stain is a polychromatic stain solution (Giemsa, Wright) containing methylene blue and eosin. These basic and acidic dyes induce multiple colors when applied to cells. Methanol acts as a fixative and as a solvent. The fixative does not allow any further change in the cells and makes them adhere to the glass slide. The basic component of white cells (i.e., cytoplasm) is stained by acidic dye and they are described as eosinophilic or acidophilic. The acidic components (e.g., nucleus with nucleic acid) take up blue to purple shades of the basic dyes and they are called basophilic. The neutral components of the cell are stained by both the dyes.

PACKAGE CONTENTS

Description	Catalogue Number	Quantity
AG-Giemsa Stain	AG/Stain/GM/22/01	200 mL

STORAGE & STABILITY

Giemsa stain should be stored at 15-25°C in their original containers. Product stored under these conditions will be stable until the expiry date shown on the product label.

TYPES OF SPECIMENS

Anticoagulated Blood specimens and blood smears

SPECIMEN COLLECTION & HANDLING

- Peripheral blood samples on clean glass slide may be collected.
- If Anticoagulated blood to be preferred use K_3 EDTA.
- Avoid Heparin as Anticoagulant.
- Make smear immediately after blood collection.

DIRECTIONS/ PROCEDURE:

1.Estimate the amount of 10% Giemsa working solution required for the number of slides to be stained. Each slide requires approximately 3 mL of stain to cover it. Prepare the 10% Giemsa working solution stain immediately before use, as mentioned below.For 10 ml of 10% Giemsa working solution;

1-1. Place 9 mL buffered water, pH 7.2, into a clean beaker or tube.

1-3. Use Whatman No.1 filter paper to filter the provided Giemsa Stain. Using a clean, dry pipette, add 1 mL of filtered Giemsa stain to the buffered water to prepare the Giemsa working solution.

1-4. Prepare the Giemsa working solution just before staining the blood film(s), and use it within a maximum of 15 minutes of preparation. Discard any unused stain.

2. To fix the thin film, preferably use methanol spray fixative or dip the slide in a beaker containing methanol for 2secs. Avoid contact between the thick film and methanol, as methanol and its vapors will quickly fix the thick film and interfere with hemolysis of the thick film.
3. Place the slides on a tray or drying rack. Allow the methanol-fixed thin smear to dry completely in air (approximately 5-8 min) by placing the slides on a flat surface. Never let the slide dry in a vertical position with the thin film down, as this may lead to fixing of the thick film by methanol vapor.
4. Place slides for staining blood films face down if using a curved staining tray or facing up if using a staining rack.
5. Pour the stain gently between the slide and the staining tray if staining face down, until each slide is covered with stain, or gently pour the stain onto the top of slides lying face upwards on a staining rack.
6. Set the timer to 15–20 min and allow the blood films to stain. The exposure time should be determined previously by testing the batch of stock staining solution used. A technical experience with the stain in use will help indicate the time required for good staining.
7. At the end of the staining time, remove each slide individually. Gently flush the stain from the slide by adding drops of buffered water (pH 7.2) until all the stain has been washed away. Do not pour the stain directly off the slides, as the metallic green surface scum will stick to the film, spoiling it for microscopy.
8. When the stain has been washed away, place the slide in the drying rack film side downwards, or in a vertical position with the thick film down to drain and dry. Ensure that thick films are not scraped against the edge of the rack.
9. Discard the remaining 10% Giemsa solution if it is over 15mins from the time of preparation.

INTERPRETATION OF THE RESULTS:

Observe the stained smear under 100x oil immersion. The cells and other components can be differentiated as follows;

- Red blood cells: Mauve-pink
- Neutrophils: Reddish purple nuclei with pink cytoplasm
- Eosinophils: Purple nuclei, faintly pink cytoplasm and red to orange granules.
- Basophils: Purple nuclei, blue coarse granules.
- Lymphocytes: Dark blue nucleus with light blue cytoplasm.
- Monocytes: Pink cytoplasm with a purple color nucleus.
- Platelets: Violet to purple color granules.
- Nuclei of host cells: Dark purple
- Nuclei of WBCs: Dark purple
- Cytoplasm of host cells: Pale blue
- Cytoplasm of white cells: Pale blue or grey-blue
- Melanin granules: Black green
- Bacteria: Pale or dark blue

- Chlamydia trachomatis inclusion bodies: Blue-mauve to dark purple depending on the stage of development
- Borrelia spirochetes: Mauve-purple
- Yersinia pestis coccobacilli: Blue with dark stained ends (bipolar staining)
- Malaria parasite: Malaria parasites have a red or pink nucleus and blue cytoplasm. If P. vivax is seen, the Schüffner dots are seen as an even carpet of pink dots in the cytoplasm of red blood cells. If P. falciparum is observed, Maurer clefts will be seen as unevenly distributed, coarse bodies in the red cell cytoplasm.

LIMITATION:

1. Only experienced personnel should carry out the interpretation of stained slides
2. Read prepared slides as soon as possible after staining. Failure to do so may affect the results.
3. Caution should be exercised when examining thick blood films as clotted cells and blood platelets may be confused with malarial parasites

WARNING & PRECAUTIONS:

- For *In vitro* Diagnostic Use only.
- For professional use only.
- Directions should be read and followed carefully.
- Do not use beyond the stated expiration dates.
- Safety precautions should be taken in handling, processing and discarding all clinical specimens.
- Dispose of all material in accordance with local regulations.

SYMBOLS:

Date of manufacture	Use-by-date	Do not use if package is damaged	Manufacturer
Batch Code	Refer to the instructions	ISO	GMP

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