Hematology Stains
REF: HS003 Giemsa
REF: HS004 May-Grünwald

Store at 15-30°C

Giemsa - May-Grünwald

Intended use
The Giemsa and May-Grünwald stains are Romanowsky stains and are used for the differentiation of blood cell types in human peripheral blood smears and bone marrow. Some authors recommend the use of these stains for the staining of parasites in blood.
For *in vitro* diagnostic use only.
For professional use only.

Clinical significance
The information gathered from the examination of blood smears is extremely important. Stained blood smears furnish the best means of studying the morphology of blood cells and parasites. In this way they can give information about blood diseases (e.g. anemia, leukemia) that alter the number, aspect, size or shapes of erythrocytes, leukocytes or platelets.

Principle
Romanowsky stains like Giemsa, May-Grünwald and Wright are constituted by Methylene blue and its oxidized forms (basic stains) as well as Eosin (acid stain). The basic stains bind to the acidic components of cells (nucleic acids, basophilic granules and acid proteins), thereby staining them in a red-purple color. Acid stains such as Eosin stain the basic components like hemoglobin and eosinophilic granules.
The ratios between Methylene blue and its oxidized forms and between the basic stains and Eosin determine the intensity of the staining and the blue shading. These ratios are characteristic for each Romanowsky stain.

Reagent composition
Giemsa (1 x 500 ml):	
Giemsa's Eosin-Methylene blue	7,0 g/l
Methanol	50%
Glycerol	50%
May – Grünwald (1 x 500 ml):	
May-Grünwald's Eosin-Methylene blue	2,7 g/l
Methanol	> 99%

Precautions
Giemsa (HS003), May-Grünwald (HS004): Contains methanol. H225 – Highly flammable liquid and vapor. H301+H311+H331 – Toxic if swallowed, in contact with skin or if inhaled. H370 – Causes damage to organs. P210: Keep away from heat, hot surfaces, open flames and other ignition sources. No smoking. P260 – Do not breathe dust/fume/gas/mist/vapors/spray. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P308 +P311 – IF exposed or concerned: Call a POISON CENTER/doctor. P321 – Specific treatment. P370+P378 – In case of fire: Use the means described in point 5 of the Safety Data Sheet. P403+P233 – Store in a well-ventilated place. Keep container tightly closed.
The stains are toxic and flammable due to its methanol content. Waste disposal should be carried out according to the local regulation in force.

Preparation
May-Grünwald stain is ready for use.
The Giemsa stain has to be diluted 1/10 with phosphate buffer (3,3 - 10mM, pH 7,0-7,2) [Note 4].

Storage and stability
When stored at 15-30°C, reagents will remain stable until the expiration date stated on the label. Containers must always be kept tightly closed.
A light precipitate may form for some reagents over time. This, nevertheless, does not affect their functionality.
Avoid excessive cold, since it may cause precipitation of the stain. If this occurs, the stain should be filtered before use. Longer staining times may be required in this case due to the loss of stain by precipitation.
The working reagent (1/10 dilution) is stable for 3 hours at room temperature (≤ 25°C).

Additional material required but not provided
- General laboratory equipment
- Microscopic slides.
- Device for sample staining (manual or automated)
- Phosphate buffer (3,3 - 10 mM, pH 7,0 – 7,2) [Note 4]
- Methanol
- Microscope

Samples
Blood films, air dried. It is recommended that the films are homogeneous and thin to obtain a better fixation of the dye without overstaining.
Staining should be performed within the two hours following the preparation in order to obtain good results. Old smears may stain irregularly.
The ideal sample is capillary blood, but if venous blood is used then EDTA should be used as anticoagulant. The use of heparin is not advised.
Handle the samples carefully due to their potentially infectious nature.

Procedure
Giemsa:
1. Just prior to use, dilute the stain (Normal Giemsa) 1/10 in phosphate buffer (pH 7,0 – 7,2).
2. Fix the air-dried blood film with methyl alcohol for 3 min.
3. After this time, decant the methyl alcohol. Without any prior washing, cover the slide with the diluted stain and allow it to stand for 8-20 min, depending on the desired staining intensity.
4. Wash abundantly with phosphate buffer (pH 7,0 – 7,2), and let air-dry.
5. Examine under the microscope.

May-Grünwald – Giemsa:
1. Cover the air-dried blood film without fixing with a known volume of May-Grünwald stain (approximately 2 ml), and allow it to stand for 3 min.
2. Pour off the stain by tilting the slide, and without any prior washing, cover the slide with Giemsa stain which has been recently diluted 1/10 with phosphate buffer (pH 7,0 – 7,2).
3. After 8-20 min, wash with phosphate buffer (pH 7,0 – 7,2) and let air-dry.
4. Examine under the microscope.

Interpretation of the results
Erythrocyte:	pink or pink orange.
Platelets:	pale violet or purple.
Neutrophils:	dark violet nucleus. Pink cytoplasm with red-violet granules.
Eosinophils:	Violet nucleus. Blue cytoplasm and red or orange-red granules.
Basophils:	Dark blue or purple nucleus. Purple granules, almost black.
Lymphocytes:	Purple nucleus. Sky blue cytoplasm.
Monocytes:	Very pale violet nucleus. Sky blue cytoplasm.

The stated results are for guidance. Color intensity and shades could change depending on pH of buffer used. In general, the color becomes more reddish when pH decreases.

Quality Control
The use of QC samples is recommended in order to assess the appropriate staining of the sample components. Each laboratory should establish its own QC scheme and corrective actions if the controls do not fulfil the established criteria.
We recommend to follow the QC practices defined by the CLSI.

Limitation of the test
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Notes
1. The staining time (8-20 minutes) will depend on the desired color intensity which will vary according to the user's criteria. The color intensity is directly related to the staining times. The stained sample must be kept away from moisture in order to obtain optimal results.
2. Each user may apply different versions of this procedure, both manual and automated, adapting it to their standard method.
3. We recommend not to use tap water for rinsing, because tap water has variable pH, salt content and chlorine content. These components can affect the staining results and could not be repetitive from day to day.
4. The concentration of the buffer is very important. A higher concentration can impair or inhibit the coloration.

Bibliography

1. Clark, G., Staining procedures (1981), 4thed. Williams & Willkins.
2. Krafts Woronzoff-Dashkoff, Kristine, Clinics in Laboratory Medicine. Vol 22 (2002).
3. Biological Stains (1977) 9th ed. Ed. By R.D. Lillie; Williams & Wilkins.
4. CLSI Guidelines and Standards, CLSI, Wayne, P.A

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