GIEMSA SOLUTION

Used for staining in hematology, cytology and staining sections of hematopoietic organs in histopathology

INSTRUCTIONS FOR USE

Introduction

Polychromatic Romanowsky dyes are a standard in hematology of blood smears and bone marrow. Various sorts of Romanowsky dyes (Giemsa, May-Gruenwald, Leishman, Wright, Jennner and others) contain different ratios of methylene bluing reagent used as the cation component (and the reagent-related thiazine dyes, such as azure B) and eosin Y as the anion component. Cation and anion components interaction creates a well known Romanowsky effect that cannot be achieved if each component is being used individually. Purple color indicates the effect’s presence. Staining intensity depends on the azure B content, as well as azure B to eosin Y ratio, while a few other factors affect the result of staining: working solution pH value and buffer solution, fixation method and dye exposure time. BioGnost's Giemsa solution is used for differentiation of nuclear and/or cytoplasmatic morphology of lymphocytes, monocytes, granulocytes (neutrophils, eosinophils, basophils), thrombocytes and erythrocytes. There are various methods of using the Giemsa solution, and the so-called Pappenheim method is one of the most commonly used ones. The method is essentially the May-Gruenwald Giemsa method combined with the May-Gruenwald solution that stains cytological material (peripheral blood smears, cytodiagnostic puncture aspirates, diarrhea or secretion cells) or hematopoietic organs’ sections. Along with the Pappenheim method, the Giemsa solution is commonly used for chromosomal aberrations detection in cytogenetics.

Product description

- GIEMSA SOLUTION - solution of eosin, methylene bluing reagent and azure dyes in methanol and glycerol with added stabilizer.

Other sections and reagents that may be used in staining:

- Fixatives such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agents, such as BioClear xylene or a substitute, such as BioClear New agent on the aliphatic hydrocarbons basis
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE, VitroGnost COLOR or one of more than 30 models of BioGnost's
- BioGnost's histology acetic acid to 99.9 ml of distilled/demineralized water.
- Fixative, such as BioGnost's Histanol M
- BioGnost's Immersion oil
- BioGnost's Buffer tablets, pH 6.8 or 7.2

Preparation of solutions

Buffer solution, pH 6.8
Dissolve 1 pH 6.8 buffer tablet in 1 liter of distilled water while stirring.

Note: During the staining process it is possible to use pH 7.2 buffer solution or a combination of pH 6.8 and 7.2 buffer solutions, and the process's results can differentiate in shift toward red or blue on the color spectrum.

BioGnost’s Giemsa solution (standard method)

1. Let the smear air dry
2. Fix previously dried blood smears by immersing them in methanol (Histanol M)
3. Immerse the fixed section into the working Giemsa solution
4. Let the smear air dry
5. Rinse the smear in the pH 6.8 buffer solution - two exchanges
6. Rinse the smear in pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.
7. Immerse the fixed section into the working Giemsa solution
8. Let the smear air dry
9. Rinse the smear in the pH 6.8 buffer solution - two exchanges
10. Rinse the smear in pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.
11. Immerse the fixed section into the working Giemsa solution
12. Let the smear air dry
13. Rinse the smear in the pH 6.8 buffer solution - two exchanges
14. Rinse the smear in pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.
15. Immerse the fixed section into the working Giemsa solution
16. Let the smear air dry
17. Rinse the smear in the pH 6.8 buffer solution - two exchanges
18. Rinse the smear in pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.
19. Immerse the fixed section into the working Giemsa solution
20. Let the smear air dry
21. Rinse the smear in the pH 6.8 buffer solution - two exchanges
22. Rinse the smear in pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.
23. Immerse the fixed section into the working Giemsa solution
24. Let the smear air dry
25. Rinse the smear in the pH 6.8 buffer solution - two exchanges
26. Rinse the smear in pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.
27. Immerse the fixed section into the working Giemsa solution
28. Let the smear air dry
29. Rinse the smear in the pH 6.8 buffer solution - two exchanges
30. Rinse the smear in pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.
31. Immerse the fixed section into the working Giemsa solution
32. Let the smear air dry
33. Rinse the smear in the pH 6.8 buffer solution - two exchanges
34. Rinse the smear in pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.
35. Immerse the fixed section into the working Giemsa solution
36. Let the smear air dry
37. Rinse the smear in the pH 6.8 buffer solution - two exchanges
38. Rinse the smear in pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.
39. Immerse the fixed section into the working Giemsa solution
40. Let the smear air dry
41. Rinse the smear in the pH 6.8 buffer solution - two exchanges
42. Rinse the smear in pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.
43. Immerse the fixed section into the working Giemsa solution
44. Let the smear air dry
45. Rinse the smear in the pH 6.8 buffer solution - two exchanges
46. Rinse the smear in pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.
A4) Blood smear staining procedure using May-Gruenwald Giemsa (Pappenheim) perioperative method

1. Let the smear air dry
2. Apply May-Gruenwald solution to the dried smear
3. Rinse the smear in pH 6.8 buffer solution.
4. Apply working Giemsa solution to the smear
5. Rinse the smear in pH 6.8 buffer solution.

Note: If necessary, apply a smaller volume of the buffer solution on the slide in order to thoroughly remove the excessive dye and to make the stained structures clearly visible. Rinse the solution after 10-30 seconds.
6. Air dry the preparation

Result (pH 6.8)
Nuclei - purple to violet
Lymphocyte plasma - blue
Monocyte plasma - grey-blue
Neutrophil granule - light violet
Eosinophil granule - red
Basophil granule - dark violet to black
Thrombocytes - violet
Erythrocytes - reddish

Preparing the histological slides and solutions for the Giemsa solution staining (bone marrow biopsy, illium biopsy)
- Fixate the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100).
- Decalcify the sample by immersing it into a mild decalcifying agent (OsteoSens). Keep it immersed for 6 hours.
- Cut the sample carefully into small slices (9-20 µm). If necessary, treat it again with a decalcifying agent (OsteoSens) for 20 min.
- Clear the sample with intermediate; in xylene (BioClear) or in a xylene substitute (BioClear New).
- Infiltrate and embed the sample in paraffin (BioWax Plus, BioWax 52/54, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 3-4 µm and place them on a VitroGnost glass slide.

B) Histological slides staining procedure using Giemsa solution

1. Deparaffinize the section using xylene (BioClear) or a xylene substitute (BioClear New), then rehydrate the section through series of descending alcohol solutions (Histanol 100, Histanol 95, Histanol 80 and Histanol 70).
2. Rinse the section with distilled/demineralized water
3. Stain the section using Giemsa solution until it is optimally stained
   Note: Use undiluted Giemsa solution instead of the working solution in this step
4. Differentiate the section using 0.1% solution of acetic acid
5. Rinse the section with distilled/demineralized water
6. Dehydrate the section through three exchanges of isopropyl alcohol (Histanol IP)
7. Clear the section through two exchanges of xylene (BioClear) or a xylene substitute (BioClear New) immediately after clearning apply an appropriate BioMount medium for covering/mounting on the section. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New. Cover the section with a VitroGnost cover glass.

Results
Nuclei - blue
Collagen, osteoid - light blue
Eosinophil granules - red
Acidophilic mucopolysaccharide, mastocytes, cartilage matrix - red-purple
Acidophilic substances - orange-red

Note
Time periods of staining processes are not entirely standardized and they approximate correspond to clinical and laboratory practical experience. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

Preparing the sample and diagnostics
Use only appropriate instruments for collecting and preparing the samples. Process the samples with modern technology and mark them clearly. Follow the manufacturer's instructions for handling. In order to avoid mistakes, the staining procedure and diagnostics should only be conducted by authorized and qualified personnel. Use only microscope according to standards of the medical diagnostic laboratory.

Safety at work and environmental protection
Handle the product in accordance with safety at work and environmental protection guidelines. Used solutions and out of date solutions should be disposed of as special waste in accordance with national guidelines. Reagents used in this procedure could pose danger to human health. Tested tissue specimens are potentially infectious. Necessary safety measures for protecting human health should be taken in accordance with good laboratory practice. Act in accordance with signs and warnings notices printed on the products label, as well as in BioGnost's material safety data sheet.

Storing, stability and expiry date
Keep the Giemsa solution in a tightly closed original package at temperature between +15°C and +25°C. Do not keep in cold places, do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the products label.

References