

Giemsa: The Universal Diagnostic Stain

Shalmica Jackson, PhD¹; Daniela Grabis, BS²; Caroline Manav, BS²

¹MilliporeSigma, St. Louis, MO, USA; ²Merck KGaA, Darmstadt, Germany

Introduction

Giemsa is a versatile polychromatic stain, which is suitable for staining a diverse range of specimens. In the early 1900s, Gustav Giemsa designed the Giemsa stain to detect parasites such as malaria and *Treponema pallidum* in blood smears. He developed a “secret” oxidation process using a unique mixture of methylene azure, methylene blue, and eosin, with glycerol added as a stabilizing agent.

Microorganisms such as *Histoplasma*, *Leishmania*, *Toxoplasma*, and *Pneumocystis* can also be detected with Giemsa, and in gastric tissues *Helicobacter pylori* (*H. pylori*) appear thin and distinctly blue. Giemsa’s stain is frequently used for diagnostic purposes in hematology to differentiate nuclear and cytoplasmic morphology of platelets, RBCs, WBCs, and parasites. It is frequently used in combination with other dye solutions: May-Grünwald’s solution for Pappenheim (MGG) and Wright-Giemsa. The resulting stain can vary depending on the influence of fixation, staining times, and the pH values of the solutions or buffers.

The Giemsa stain was adapted to histology due its unique staining of chromatin, nuclear membranes, and cytoplasmic elements. The staining obtained in tissue sections is more variable than in smears because of the different steps required (differentiation, dehydration, clearing). The color of the various cellular components is influenced by pretreatment of the specimen material. Clinical cytological material like urine sediment, sputum, smears from fine needle aspiration biopsies (FNAB), rinses, and touch preps are also used as starting material for the Giemsa stain.

The field of cytogenetics was expanded with the discovery that metaphase chromosomes digested with trypsin could be stained with Giemsa to reveal AT-rich and GC-rich regions (G-banding patterns). The unique advantages of the Giemsa stain were also leveraged to establish the Kiel classification of lymphomas due to its preferential staining of hematology tissues.

This poster demonstrates the versatility of the Giemsa stain due to its use in a wide variety of applications, including hematology, histology, cytology, bacteriology, and cytogenetics.

Giemsa Applications

Blood	Lavages
Bone marrow	FNAB (fine needle aspiration biopsy)
Sputum	Touch preps
Urine	Paraffin sections (e.g. stomach biopsy for detection of <i>H. pylori</i>)
Body effusions	Lymph nodes
Spleen samples	Tonsils
Cytogenetics	Karyotyping

Materials and Methods

Reagents: Giemsa’s azure methylene blue solution; methanol; 2-propanol; buffer tablets (pH 6.4, 6.8 and 7.2) acc. to WEISE; NaCl tablets; trypsin; glacial acetic acid, acetone; 2-propanol; Hank’s Balanced Salt Solution; Neo-Clear® xylene substitute. Neo-Mount® anhydrous mounting medium; OSTEOSOFT® mild decalcifier-solution. All reagents used were from MilliporeSigma, Burlington, MA.

Specimens: Blood smears, lymph node sections, lymph node touch preps, bone marrow biopsies, gastric mucosa infected with *H. pylori*, iliac crest biopsies, lung touch prep including squamous cell carcinoma, and Hodgkin’s lymphoma specimens were selected. Fixed bone marrow biopsy material was first decalcified for 18 hours, prior to histoprocessing. Malaria-infected blood smears and *Trypanosoma*-infected blood smears were a kind gift from the University of South Africa. All samples were analyzed in triplicate.



Blood smears: Samples were air-dried thoroughly prior to staining. Concentrated Giemsa solution was diluted with buffer solution (1 pH buffer tablet per 1L distilled water) and filtered prior to use. Thick smears were generated with 6 μ l blood confined within a small circle, and thin smears were made using 2 μ l of blood spread out with a feathered edge. Thin smears were fixed with methanol to maintain the RBC morphology to aid in the identification of *Plasmodium*.

Paraffinized biopsy specimens: Pretreatment of bone marrow and iliac crest biopsy materials using OSTEOSOFT® mild decalcifier solution. Slides were deparaffinized and rehydrated in a descending alcohol series. Concentrated Giemsa, undiluted and filtered, was used for staining.

Clinical and intraoperative smears, lymph node touch preps, lung tumor touch preps: Samples were air-dried and fixed in methanol for 1 min. For fast Giemsa staining, Giemsa solution was used undiluted and filtered prior to use. Slides were stained with concentrated Giemsa for 1 min followed by 2 x 1 min washes with pH 6.8 buffer solution.

Instrument: Midas® III-Plus Automated Stainer for hematology and bacteriology. Direct deionized water supplied at a flow rate of 1500 mL/min. Slides were dried at 65°C. Diluted Giemsa solution was used.

G-banding: Metaphase spreads were prepared and subjected to trypsin digestion to remove chromosomal proteins then extensively rinsed with 0.9% NaCl. Slides were stained with diluted Giemsa in acetone for 5 min followed by rinses in pH 6.8 buffer solution.

Results

Figure 1: Hematology staining of a blood smear.

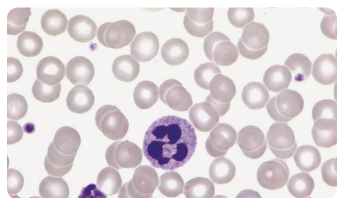
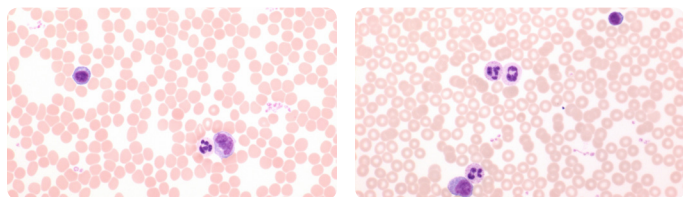
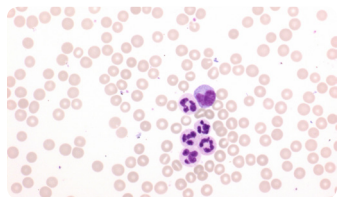


Figure 2: Hematology staining of blood smears at pH 6.4 (A), 6.8 (B), and 7.2 (C).



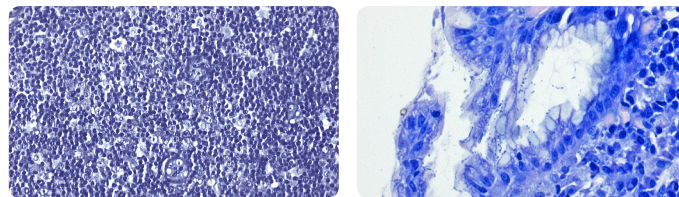
A.

B.



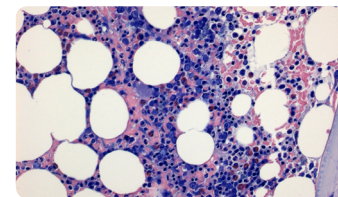
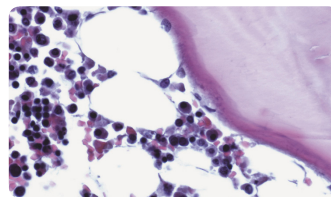
C.

Figure 3: Histology staining of lymph node (A), gastric mucosa infected with *H. pylori* (B), and Iliac crest biopsies (C & D).



A.

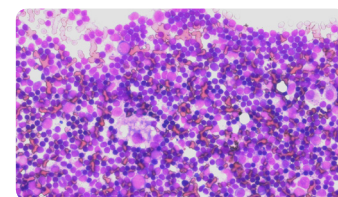
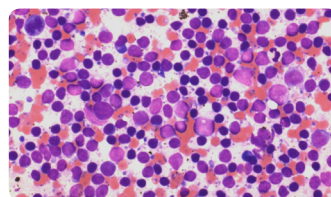
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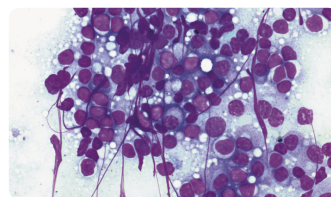
D.

Figure 4: Cytology staining of lymph node touch prep with Giemsa Fast Stain (A), lung touch prep (squamous cell carcinoma) with Giemsa Fast Stain (B), and Hodgkin's lymphoma (C).



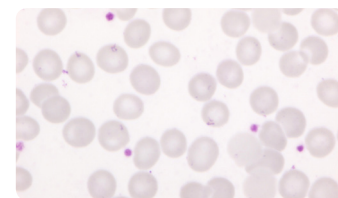
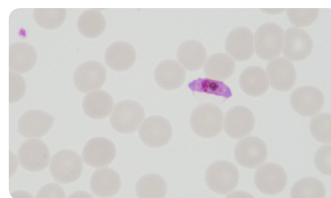
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B.



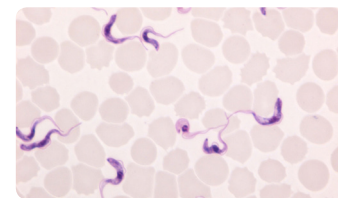
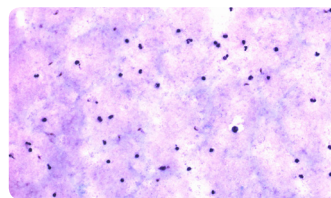
C.

Figure 5: Bacteriology staining of infected blood. Malaria-infected blood-Gametocyte, thin smear (A) *Plasmodium*, thin smear (B) and thick smear (C), and *Trypanosoma brucei*-infected blood (D).



A.

B.



C.

D.

Figure 6: Midas® III-Plus Automated Stainer (A). Blood smears stained on the stainer at pH 6.4 (B), 6.8 (C), and 7.2 (D).

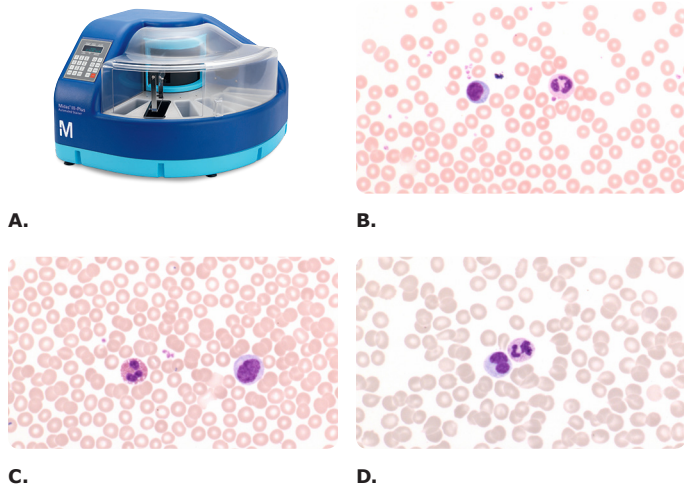
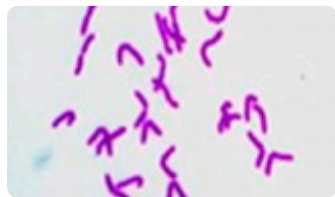


Figure 7: G-banding for karyotype analysis.



Discussion

Giemsa is classified as a versatile stain primarily due to its unique formulation. Its utility is well established in hematology for blood and bone marrow specimens, bacteriology, clinical cytology specimens, histological biopsies, and tumor samples.

Giemsa staining is highly influenced by pH level. At low pH levels, erythrocytes appear red, and at higher pH levels, they appear more blue-gray to deep violet (Figures 1 and 2). More acidic pH levels provide for more chromatin staining and less cytoplasmic staining, conversely, more alkaline pH levels enhance the visibility of denser nuclei and increased cytoplasmic staining.

In histological sections, cell nuclei can range from deep purple to dark blue, collagen a pale blue, acidic mucopolysaccharides a reddish-violet, other acidic cellular materials orange-red, and in the case of *H. pylori*, blue to dark blue (Figure 3). With method optimization, typical and atypical cellular patterns can be demonstrated in a variety of tissues.

Classical Giemsa staining takes between 20 to 25 minutes, which makes it less suitable for intraoperative use. However, Giemsa Fast Staining for clinical use on lymph node and tumor touch preps takes less than 5 minutes by using a stable concentrated stock of Giemsa (Figure 4). Giemsa Fast Staining is a viable alternative to H&E staining for time-sensitive intraoperative results. Traditional Giemsa staining is also used to routinely stain clinical cytology specimens, such as Hodgkin's lymphoma.

Giemsa is the prototypical stain used to detect malaria and *Trypanosoma*-infected blood (Figure 5). *Plasmodium falciparum* gametocytes and mature trophozoites can be detected using thin and thick smears, respectively. WBCs, platelets, and remnants of RBCs are also visible with Giemsa staining on thin and thick smears. Thin smears are fixed with methanol to maintain RBC morphology and to aid in identification. In contrast, RBCs are not visible on thick smears due to the dehemoglobinization process. The thick smear acts as a concentrated blood smear to aid in detecting low parasitemia and the lysing of the RBCs is critical.

Reproducible results can be obtained when using Giemsa manually in jars and racks (Figure 1 and 2) and when using the stain in automated slide stainers (Figure 6).

Traditional G-banding of metaphase chromosomes allows identification of individual chromosomes and detection of gross chromosomal anomalies and abnormal chromosome structures (Figure 7). G-banding is the most characterized technique that produces characteristic banding patterns.

Summary

For 100 years, the Giemsa stain has proven to be the preferred microscopic stain worldwide. This universal special stain is used in a wide variety of applications including hematology, histology, cytology, bacteriology, and cytogenetics.

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