

TITLE: Manual Procedure for Giemsa Plus Stain Kit

I. PURPOSE

Remel Giemsa Stain Kit is a rapid, differential stain kit intended for use in the detection and identification of blood and tissue parasites. It is a supplemental method to the traditional Wright or Wright-Giemsa staining.

II. SCOPE

To provide Hematology-Chemistry Laboratory, Hematology department staff with guidelines and procedures on how to perform manual staining procedure using Remel Giemsa Plus Stain Kit.

III. PRINCIPLE

Smears are fixed using the Giemsa Plus fixative. Slides are immersed in Giemsa Plus reagent A and in Giemsa Plus Reagent B, individually, to differentially stain specific cellular components. Reagent A and B are anionic and cationic dyes respectively. These charged dye molecules form ionic bonds with opposite charged site of proteins. The cellular components stain either basophilic (blue) or eosinophilic (red). The color intensity can be varied by adjusting the staining time in each reagent.

IV. RESPONSIBILITIES

Roles	Responsibilities
Quality Assurance	Supports the process including provide leadership and/or assistance in support of the process. Review and approval of procedure (site dependent).
Medical Director	Supports the development of the document. Review and approval of the document.
Management	Review and approve the document. Ensure that procedure is followed.
Laboratory Technical staff	Follows procedure.

V. ACRONYMS/DEFINITIONS

URMC	University of Rochester Medical Center
SMH	Strong Memorial Hospital

VI. SPECIMENS

- A. EDTA blood specimen or 5cc of whole blood in a 50 ml flask (blood must be fresh and collected during the patient's chill or fever spike).

VII. QUALITY CONTROL

- A. The patient smear can serve as quality control to verify the efficacy of the staining reagents. If the leukocytes and erythrocytes exhibit typical colors, parasites can be expected to stain correctly.
- B. In addition, a smear made from a patient blood specimen (previously identified as positive) may be included to verify differential staining characteristics and compare with specimen stain results.
- C. To prepare quality control slides from a patient's blood:
- Positive QC slide: Choose a patient blood specimen, anticoagulated with EDTA, that has enough parasites so that at least one is found in every 2 to 3 fields.
 - Negative QC slide: Choose a previously tested patient blood sample that test negative for blood parasites.
 - Make as many thin smears as possible, preferably within one hour after the blood was drawn from the patient.
 - Allow the smears to dry quickly, using a fan or blower at room temperature.
 - Label appropriate as "Pos for BP" or "Neg for BP".
 - Fix the smears in absolute (100%) methanol; allow them to dry.
 - Place them, touching front to back, in a box without separating grooves.
 - Label the outside of the box with the contents, date and "Blood Parasite control slides."
 - Store at -70°C (or colder) until needed for use.
- D. Just before use, remove the smear from the box and allow the condensation to evaporate; label the slide with the present date. The smear is now ready for staining since it was previously fixed.
- E. If aberrant quality control results are noted, patient results should not be reported.
- F. Competency assessment test for blood parasites is to be performed on the medtraining.org website. A minimum score of 70% must be obtained to be considered passing.

- G. Any major problems with competency will be discussed on an individual basis. If the technologist reports a value(s) outside the acceptable range, that technologist receives a "deficiency". Two consecutive deficiencies by the same technologist will result in remedial action.

VIII. SPECIAL SAFETY PRECAUTIONS

- A. All patient specimens should be considered potentially infectious and must be handled with precautions used for human blood, as described in CDC (Center for Disease Control) recommendations and in compliance with the Federal OSHA (Occupational Safety and Health Administration) Blood-borne Pathogen Standard, 29 CFR (Code of Federal Regulations) part 1910.1030. Follow specimen handling as outlined by the Laboratory Safety Policy, SH.CP.AU.gen.0005.
- B. Giemsa Plus Fixative
CAUTION: POISON! May be fatal or cause blindness if swallowed.
Flammable! Keep away from heat, sparks or flame.
VAPOR HARMFUL! Causes eye irritation.
- C. Giemsa Plus Reagent A
CAUTION: Contains Formaldehyde. Suspected Human carcinogen.
- D. Remel Giemsa Staining Kit: This product is for In Vitro diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers and media after use. Directions should be read and followed carefully. Refer to Safety Data Sheets for additional information on reagent chemicals.

VIII. MATERIALS

- A. Equipment
 - 1. Microscope with 100X Oil immersion lens
- B. Supplies
 - 1. Coplin jars with lids
 - 2. Microscope slides
 - 3. Immersion oil
 - 4. Positive control slide
- C. Reagents
 - 1. Giemsa Plus reagents are ready to use. No additional preparation is required. Pour reagents into Coplin jars or staining jars with covers.

- a. Giemsa Plus Fixative: 2.0 mg/ml Malachite Green in Methanol
CAUTION: POISON! May be fatal or cause blindness if swallowed.
Flammable! Keep away from heat, sparks or flame.
VAPOR HARMFUL! Causes eye irritation.
 - b. Giemsa Plus Reagent A: 0.1% Eosin in buffer and 0.1% Formaldehyde, pH 6.8-7.2
CAUTION: Contains Formaldehyde. Suspected Human carcinogen.
 - c. Giemsa Plus Reagent B: 9.91 g/l Thiazine dye mixture in buffer pH 6.8-7.2
2. Store product in its original container at room temperature until used. Keep container tightly closed during storage.
 3. Giemsa Plus products should not be used if:
 - a. The color has changed.
 - b. The expiration date has passed.
 - c. There are other signs of deterioration.

IX. PROCEDURE

- A. Prepare slides using same step/action found in steps IX – A through G of Blood Parasite Smear procedure (see SH.CP.AU.hem.0005).
- B. Dip slides in Giemsa Plus Fixative 5 times, 1 second per dip. Drain excess.
- C. Dip in Giemsa Plus Reagent A 5 times, 1 second per dip. Drain excess.
- D. Dip in Giemsa Plus Reagent B 5 times, 1 second per dip.
- E. Rinse with distilled water.
- F. Allow the smear to air dry.
- G. Examine under oil immersion.

X. LIMITATIONS

- A. Giemsa Plus is an aqueous stain. The water soluble portion of the cellular components may take up the stain differently than the traditional alcohol-based Wright stain, resulting in basophils which appear “washed out”.
- B. If basophils are suspected in the specimen, it should be stained using a Wright stain.
- C. If an overall lighter stain is desired, decrease the number of dips in Reagent A and B to no less than 3 dips. Each dip should be 1 full second.
- D. The intensity of the stain may be altered by varying the number of dips in Reagents A and B.

1. To increase eosinophil staining, increase the number of dips in Reagent A.
2. To increase basophil staining, increase the number of dips in Reagent B.
3. To increase the overall intensity of the stain, increase the number of dips in both Reagent A and Reagent B.

XI. CALCULATIONS

NA

XII. INTERPRETATION

A. Four species of Plasmodium may be found in man:

1. *P. falciparum*: Chills and fever recur at 48- hour intervals. Usually, only rings and/or crescents (gametocytes) are seen in the smear. Double chromatin dots in the rings are characteristic.
2. *P. vivax*: Chills and fever recur at 48- hour intervals. All forms may be seen on the smear, including Schuffner's Granules.
3. *P. malariae*: Chills and fever recur at 72-hour intervals. Round gametocytes or all six forms are seen.
4. *P. ovali*: A very rare, benign form. Oval presegmented forms (early schizonts) seen.
5. The following forms of the parasites can be seen in the blood film: early rings, growing trophozoites, early schizonts, mature schizonts, late segmenters, male gametocytes and female gametocytes. In all forms, the cytoplasm stains blue and the chromatin stains red using Modified Wright-Giemsa stain. All six of the above forms may appear simultaneously in films from patients with *P. vivax* or *P. malariae*.
6. Refer to appropriate reference materials or Blood Parasite procedure for more information (SH.CP.AU.hem.0005).

XIII. RESULT REPORTING

- A. The Giemsa Plus stain is used as an aid in differentiating between the different species of Plasmodium. Schuffner's dots and Maurer's dots are more readily seen when stained with Giemsa Plus than when the slide is stained with Wrights or Wright-Giemsa.
- B. Reference range: Negative

XIV. TRAINING

Staff is trained by a laboratory designated trainer and a training record is completed and signed by both trainer and staff (trainee).

XV. REFERENCES

- A. Garcia, L.S. and D.A. Bruckner, 1997. Diagnostic Medical Parasitology. 3rd ed. ASM Press, Washington, DC.
- B. Clinical and Laboratory Standards, Institute (CLSI), 2000. Laboratory Diagnosis of Blood –Borne Parasitic Diseases; Approved Guideline. M15-A. CLSI, Wayne PA.
- C. Isenberg, HD, 2004. Clinical Microscopy Procedures Handbook. 2nd edition, Vol 2, ASM Press Washington DC.
- D. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller, 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, DC.
- E. New York State Department of Health, Clinical Laboratory Standards of Practice, Part 2 – Specialty requirements, June 2017, p.134.
- F. <https://www.cdc.gov/dpdx/diagnosticprocedures/blood/staining.html>

