Modified Giemsa Stain for the Detection of Parasites in Blood (Malaria Stain)

Purpose	During some stages in their life cycle, species of Plasmodium, Babesia, Trypanosoma, Leishmania donovani, and filaria are detectable in human blood. Plasmodium and Babesia species are found within the RBCs; trypanosomes and microfilariae, the larval stage of filariae, are found outside the RBCs; and Leishmania amastigotes are occasionally found within monocytes. Trypanosomes and microfilariae, which frequently are present in low numbers, exhibit motility in freshly collected blood smears, and this can aid in their detection. Species identification of all blood parasites are made from two types of stained blood films: a thin film and a thick film.		
Scope	Histology Technical Staff		
Materials	**Supplies**	**Reagents**	**Equipment**
	• Gloves, PPE and engineering controls • Graduated cylinders • Disposable pipets • Glass Coplin jars with lids • Volumetric/ Erlenmeyer flasks	• Phosphate Buffer (Sodium & Potassium), pH 7.0 - Storage: General • Triton X - Storage: General **(optional/ backup)** • Sodium Phosphate, DiBasic - Storage: General • Potassium Phosphate, Monobasic - Storage: General	• pH meter
Sample SOP HEME 8.5 MALP (Blood Parasites)	Four thin peripheral blood smears (routine) and four thick blood preparations are prepared by Hematology. The thick preparation slides are made by placing one drop of blood on a glass slide and then spreading the blood over the slide in a circle to the size of a nickel, then air drying well.		
Records/ Forms/ Documents needed	CBC data and order for Malaria staining provided by Hematology. QC Log for Hematology and Cytology Specimens Histology Requests/ QC ("Blue Sheet") Form for Modified Giemsa stain if additional request.		
Quality Control	Use a smear with known organisms, usually purchased in a fixed state. *Note: Occasionally, there are patient blood smears that are strongly positive for Malaria organisms. Control smears may be made, dated and used from these samples ONLY with prior Pathologist approval. These controls may be used UNFIXED (or fixed, as the purchased control slides). Unfixed blood smears for Malaria controls should be stored at room temperature, shielded from light and air (ie: a slide folder or slide box), designated as unfixed and be used within a 6 month period. **Stain is pH Sensitive. The pH of the phosphate buffer is checked and documented on the pH meter log.		
Stock Solutions	**Phosphate Buffer (Sodium & Potassium), pH 7.0** Shelf life: 1 year at room temperature Manufactured and vended by Newcomer Supply **Wright's-Giemsa Stain Solution** Shelf life: 1 year at room temperature Manufactured by EMD Chemicals, Purchased through Cardinal Health **Triton X, 10% Solution** Shelf life: 1 year at room temperature Triton X (stock).....10.0 mL Distilled water ....90.0 mL		
Working Solutions	Calibrate the pH meter prior to preparing the working buffer solution. Use the two-point pH calibration check as indicated in the manufacture guidelines, and record the readings on the		

<table>
<tr><td></td><td>pH meter form. The log and the directions for calibration are located with the pH meter in the bin labeled "pH meter".

The following solutions must be made fresh (Buffer-Stain Working Solution may be used up to four hours after preparation).

Phosphate Buffer (Sodium & Potassium) Solution, pH 7.0 (pH 7.0 ± 0.2)

**Even though this is a purchased product indicating a pH of 7.0, check/ verify pH of this solution and document pH on the pH meter form.

Phosphate Buffer - Triton X Solution
Phosphate Buffer (Sodium & Potassium) Solution, pH 7.0 .....90.0 mL
Triton X, 10% (stock) Solution (mix well).....10.0 mL

Buffer-Stain Working Solution
Phosphate Buffer- Triton X (working) Solution……..……45.0 mL
(Save the remaining solution for rinsing; see Step 2 below).
Stock Wright /Giemsa Stain…….....……5.0 mL</td></tr>
<tr><td>Procedure</td><td>• Stain 2 thin (routine) smears according to Wright/Giemsa Procedure.

• Stain 1 thick prep slide and 1 Malaria control slide according to the Modified Giemsa Procedure-Malaria (below). DO NOT FIX PATIENT SLIDE.
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Place the control slide and thick prep slide into the working Buffer-Stain Solution…… 50 minutes.
*Keep the slides and solution covered during this step to prevent stain precipitation from forming on the smears.</td></tr>
<tr><td>2</td><td>Place slides directly into remaining working Buffer-Triton X Solution to remove excess stain…… 4 minutes
*Do not overly agitate slides. Do not rinse slides in water after this step.</td></tr>
<tr><td>3</td><td>Dry off slides.</td></tr>
<tr><td>4</td><td>Air-dry in a vertical position, and coverslip with synthetic mounting media.</td></tr>
<tr><td>5</td><td>Label slides with appropriate CoPath slide labels. Review Wright stain and Modified Giemsa stained slides and document on the QC Log for Hematology & Cytology Specimens. Return slides to Hematology for review. Slides should be then routed to assigned case pathologist.</td></tr>
</table></td></tr>
<tr><td>Procedure Notes</td><td>This procedure was adapted from Detection of Parasites in Blood found in the Hematology manual. The control slides are available and purchased in a fixed state only. The patient thick prep is stained with Modified Giemsa unfixed; the red cells will lyse from the patient slide, but with little to no lysing of the control smear. (*see “Note” under Quality Control section of this procedure).

If problems arise with the Phosphate Buffer (freezing, contamination, or the pH <6.8 or >7.2, etc.), Sodium and Potassium Phosphate Buffers may be made by following the prior procedure.</td></tr>
<tr><td>Interpretation/ Reports/ Alert Values</td><td>If positive, thick smear should have extracellular and/or intracellular RBC/WBC parasitic inclusions (blue). Control slide should demonstrate parasitic inclusions with minimal or no RBC lysis.</td></tr>
<tr><td>Result Reporting</td><td>Done by Hematology and Pathologist. Staining quality control documented by Histology staff on “Heme & Cytology” QC log.
St.Paul cases are stained in Minneapolis and sent back to St.Paul for QC by main lab and then slides goes to the Pathologist in St.Paul</td></tr>
<tr><td>References</td><td>Hematology procedure manual: Detection of Parasites in Blood. 7/9/99
SOP HEM 8.5 MALP (Blood Parasites)
Ash, L.R., et al, Parasites: A Guide to Laboratory Procedures and Identification, ASCP Press,</td></tr>
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	Chicago, IL, 1987, pp 99-116		
Authorization	**Designee**	**Signature**	**Date**
	Medical Director	Dennis Drehner DO	6/3/2009
	Medical Director	Revisions/Approval: Dragos Luca MD	4/27/2010
	Medical Director	Revisions/Approval: Peter Helseth MD	5/10/2012
	Medical Director	Megan K. Dishop, MD	6/3/2015
		Megan K. Dishop, MD	7/13/2017
Annual Review	**Designee**	**Signature**	**Date**
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		Dave Slinger	4/27/2010
	Pathology Assistant	Melissa Turner, PA	5/10/2012
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	Histology Supervisor	Revisions: Prabha Chintapalli (Clarify QC Process)	10/2016
		Prabha Chintapalli	6/19/2017