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| **Prepared by:** | Nancy J. Konopka, MS, MT(ASCP) |
| **Supervisor Approval:** |  |
| **Administrative Director Approval:** |  |
| **Medical Director Approval:** | **I have reviewed this document and approve it for use.** |
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**PURPOSE:**

This procedure provides instructions for the preparation, staining and examination of Wright-Giemsa stained blood smears for the detection of blood parasites including, but not limited to: Plasmodium, Babesia, and Trypanosoma species, Leishmania donovani, and microfilariae. can be seen using Wright's-Giemsa stain. In the presence of blood parasites, slides are referred to a reference laboratory for speciation.

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| **PRINCIPLE:**  Depending on the life cycle stage, a number of parasites may be recovered from a blood specimen. The parasites can be seen using Wright's-Giemsa stain. The morphologic characteristics are best observed in thin films. Thick films allow a larger amount of blood to be examined, increasing the possibility of detecting light infections. |

**REAGENTS/SUPPLIES:**

Microscope slides

Wright's-Giemsa stain

Phosphate buffer (ph ~6.8)

Automated stainer or slidemaker-stainer

Microscope

Plastic transfer pipettes

Coplin jar

**SPECIMEN/SPECIMEN COLLECTION:**

EDTA anticoagulated venous or capillary blood is the specimen of choice.

**PROCEDURE:**

**QUALITY CONTROL:**

The stain quality of the slide-maker stainer (SMS) and stand-alone automated stainer (AS) are monitored and documented daily as per HEM 1406P (SMS) and HEM 5530P (AS).

**SAMPLE PREPARATION**

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| Step |  |
| 1 | **Thin smear preparation**  Prepare several thin smears as soon as possible after specimen collection, according to the Preparation and Examination of Peripheral Smears procedure. |
| 2  3 | **Thick smear preparation**   * Prepare four thick smears by placing two or three drops of blood on each of four glass slides. * Using the pipette tip, spread the blood on each slide in a circle over an area approximately two centimeters in diameter. * Allow drying at room temperature for 8 to 12 hours. * Place the slides in a Coplin jar and wash under cold tap water for three to five minutes to lyse RBCs. Parasites should remain adhered to the slide.   **Slide staining- all smears**  Use an automated stainer to stain thick and thin smears with Wright-Giemsa stain. |
| 4 | **Examination of thick smear**   * Examine the **thick smear** for the presence of blood parasites at 1000X total magnification. Review at least 300 fields. * Record observations on Blood Parasite worksheet. * The ghosts of RBC's near the margins of the thick smear are often intact so that cell/parasite relationships (including granules and stippling) are maintained. * **If parasites are noted** proceed to examine the thin smear for parasite identification. * **If NO parasites are noted,** proceed to examine the thin smear for parasite the possible presence of parasites. |
| 5 | **Examination of thin smear**   * Examine the **thin smear** for the presence of blood parasites at 1000X total magnification. Review at least 300 fields. * Parasites tend to be concentrated near the feathered edge and along the margins of the thin smear as a result of the spreading process. * Using available textbooks for reference, identify parasites by **genus.** It is not necessary to speciate parasites. * Record observations on Blood Parasite worksheet. |
| 7 | **Pathologist review/reference laboratory confirmatory identification**   * After examination, refer all slides to a pathologist for review regardless of presence or absence of parasites. * In the presence of blood parasites, refer specimen and/or slides to a reference laboratory for speciation. |

**SAMPLE TESTING/RESULTS REVIEW and REPORTING**

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| Step |  |
| 1 | Report results in LIS computer system according to standard procedure.   * If no parasites are seen, report: NO ORGANISMS SEEN. * If parasitic forms are present, choose the best description using F<9> lookup. NOTE: Use “OTHER” with a detailed result comment explaining when other organisms are present such as Babesia, Trypanosoma, Leishmania, etc. * Indicate pathologist review using a specimen/result comment * Presence of blood parasites is considered an alert value. Notify the physician immediately per the Critical Value documentation procedure. |

**METHOD LIMITATIONS**

The absence of parasites in one set of blood slides does not rule out a parasitic infection.

**REFERENCES**

Diagnostic Medical Parasitology. Garcia, L. and Buckner, D. ASM Press. Los Angeles, CA. 1997

Clinical Microbiology Procedures Handbook, Volume II. ASM Press. Washington, DC. 1992.

**Document History**

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