**Blood Parasites**

**Purpose:** To identify blood borne parasites on Wrights-Giemsa or Modified Giemsa-stained slides.

During some stages of their life cycle, species of Plasmodium, Babesia, Ehrlichia/Anaplasma, Trypanosome, Leishmania donovani and Filaria are detectable in human blood. Plasmodium and Babesia species are found within the RBCs; Trypanosomes and Microfilaria, the larval stage of Filaria is found outside the RBCs; Ehrlichia/Anaplasma can be found in Monocytes or Granulocytes; and Leishmania amastigotes are occasionally found within Monocytes. Species identification of blood parasites is made from thin and thick stained blood films. The thin film can be stained within a few minutes and will provide a quick diagnosis if the patient has a high degree of parasitemia; the thick film is stained in the Histology lab and will aid in the diagnosis of milder infection.

**Policy Statement:** This procedure applies to all laboratory technologists who perform Hematology testing.

**Specimen:** Whole blood Anticoagulated with potassium EDTA (K2 or K3) collected from a venipuncture or capillary method. Whole blood from a fingerstick capillary collection placed directly onto a glass slide.

* Transport to the lab at room temperature
* Blood films prepared from venipuncture blood when an anticoagulant is used **must** be prepared within one hour of collection to maintain morphology of the infected RBCs and parasites.
* Specimen should be collected before treatment begins whenever possible.
* Thin and thick smears can be made directly on clean frosted end slides at the bedside.
* Testing may be ordered/collected every 12-24 hours for several days, to ensure adequate discovery of a parasite.

**Materials:**

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| **Reagents** | **Supplies** | **Equipment** |
| Normal Saline, obtained from the Blood bank | Glass SlidesWintrobe tubesPasteur Pipette | Sysmex automated slide stainerMicroscopeCentrifuge |

**Reference Range:** Negative; No intracellular or extracellular parasites present.

**Critical Value:** The presence of any parasite is considered a critical value and should be called to the patient's caregiver immediately. Document the first name and last initial of caregiver and time called.

**Quality Control:** CAP survey slides for Blood parasite detection are performed 3X/year and reviewed by technical staff. Positive control slides are available for review.

**Procedure: Before processing sample, review order in Sunquest order entry to identify which parasite the physician is looking for. If not noted, call the patient's caregiver for clarification.**

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| **STEP** | **ACTION** |
| Slide Prep | Sample preparation for **Malaria and/or Babesia orders**:1. Prepare Thin smears:* Make 6 - 8 smears with a feather edge.
* Label frosted end with patient's last name and accession number.
* Allow to dry completely before staining 2 (two) in the Sysmex Slide Maker/Stainer. Place remaining slides in slide mailer to protect from dust.

2. Prepare Thick smears:* Place 20ml of blood on 4-6 clean slides.
* Using a plain wooden applicator stick spread the 20ml over an area the size of a nickel.
* Newsprint should be just legible through the thick prep.
* Label frosted end with patient's last name and accession number.
* Allow to dry completely without the use of heat; Place in slide mailer
 |
| Examination of smears | **Two technical staff members must immediately examine the Sysmex stained slides for at least 5 minutes.** Record all results on Blood Parasite worksheet.* Using low power, examine the side edges and feathered edge of the smear.
* Using 100x oil, examine a minimum of 300 fields (or 5 minutes) looking for extracellular and/or intracellular parasitic inclusions.
* Consult pathologists and hematology/parasitology texts to identify.
* In the event of a positive smear for Plasmodium or Babesia, call the critical preliminary result to the caregiver, and order and perform a Quantitative Blood Parasite test, see [HEM 1.2 PARQ Blood Parasites, Quantitative.pdf](http://khan.childrensmn.org/Manuals/Lab/SOP/Heme/Heme/198947.pdf)
* **Enter the results of the THIN smear into Sunquest.**
* After TWO techs have done the initial review, deliver all slides directly to Histology. They will coverslip the two Sysmex stained slides, and stain and coverslip 2 (two) of the thick smears.
* Slides delivered before 2:30 M-F will be stained and returned to Hematology for final thick smear review. Slides delivered after 2:30 M-F and on weekends will be stained the following day (or Monday) and then returned to Heme for thick smear review.

**Examine Thick smears as soon as returned from Histology:** TWO techs must **s**can the entire smear using the 10X low power objective, moving to 100X oil when needed, to look for parasites. The visual examination of the smear consists of white cell nuclei and platelet debris. Since the erythrocytes (RBCs) have been lysed and the parasites are more concentrated, the thick smear is useful for screening for parasites and for detecting mixed infections. **Enter the results of the THICK smear into Sunquest.** |

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| **STEP** | **ACTION** |
| Additional Information | Blood Parasite examination may also include scanning of the slide for **Ehrlichia/****Anaplasma or Microfilaria or Blood Flagellate.**If the provider is suspicious of Ehrlichia/Anaplasma infection they should be advised to request a PCR test. |
| Examination of smears | * Suspicion for Ehrlichia/Anaplasma would display intra-cytoplasmic bacterial aggregates (Morulae) in Monocytes or Granulocytes. E. chaffeensis is primarily found in mononuclear cells. E ewingii and E. phagocytophilia are found in granulocytes.
* Circulating microfilariae can be detected by examination of the thick smears.
* As part of the complete Blood Parasite examination, record presence or absence of these blood parasites on the worksheet.
* **Enter results in Sunquest (THICK) smear.** *See resulting section.*
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**Result Entry in Sunquest:** Result the MALP test in worksheet H2 by answering the prompts for the thin and thick smear (codes; THINS and THICK). Use the following codes for resulting;

* **NBPS:** No Blood Parasites seen. Preliminary result, final CoPath result will follow.
* **PPSP** Probable Plasmodium Species present. Preliminary result, final CoPath result will follow.
* **PBSS** Probable Babesia Species present. Preliminary result, final CoPath result will follow.
* **PMOP** Probable Morulae (Intra-cytoplasmic bacterial aggregates) present. Preliminary result, final CoPath result will follow.
	+ When Ehrlichia or Anaplasma are being ruled out, type HIDE for the thin smear result (THINS) and result for the thick smear only (THICK).
	+ Manually add the bill code: MALPE for Ehrlichia/Anaplasma.
* **PMFP** Probable Microfilaria or Flagellate present. Preliminary result, final CoPath result will follow.

\*\* In the event of a positive smear for Plasmodium or Babesia, order and perform a Quantitative Blood Parasite test. See [HEM 1.2 PARQ Blood Parasites, Quantitative](https://starnet.childrenshc.org/References/labsop/heme/heme/hem-1.2-parq-blood-parasites-quantitative.pdf)

**Final Pathologist Interpretation/Review:** Following tech review of slides, and entry of results into Sunquest, submit all of the stained slides and paperwork with tech results to the Heme pathologist for final interpretation and CoPath entry.

* + Blood Parasite worksheet with slide holder
	+ Printed interim report (IRA)
	+ Sysmex printout if available
	+ Pathologists return Blood Parasite worksheet to Heme department for filing

**Notes:**

1. Color Atlas and textbooks are available on the Hematology shelf to help identify parasites.
2. In P. malaria, and P. falciparum infections, red cells are usually normal in size. In P. vivax infections, red cells can be normal to enlarged (up to 1 1/2× to 2×) in size and may be distorted. In P. Ovale infections, red cells are oval and enlarged.
3. Babesia infections show normal sized red cells. Typically, rings are seen, and they may be vacuolated, pleomorphic or pyriform. Extracellular or tetrad-forms may also be present.
4. Some microfilariae are released into the blood at certain times of the day; W bancrofti and Brugia species are usually released between 10 p.m. and 2 a.m. (nocturnal periodicity), while L loa is released mostly from 10 a.m. and 2 p.m. (diurnal periodicity). It is therefore important to collect blood during these time periods for optimal detection sensitivity.
5. Review the Sysmex Histogram pattern of PLTF if available. Malaria will often show up as debris or black scatter lined up vertically.
6. For clinical significance details see: [Table AG – Blood Parasites: Clinical Significance](http://khan.childrensmn.org/Manuals/Lab/SOP/Heme/Res/200709.pdf)

**Historical Record:**

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| **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Laura Rachford | 07/17/98 | Updated, new format |
| 2 | Laura Rachford | 07/09/99 | Updated to include Histology directions |
| 3  | Laura Rachford | 04/02/02 | Updated, more diagnostic info |
| 4 | Al Quigley | 12/31/08 | Updated |
| 5 | Al Quigley | 01/12/10 | Updated to include the resulting of a CBC and RETIC whenever an MAL is ordered |
| 6 | Al Quigley | 07/10/10 | Updated for preparation of thick smear |
| 7 | Al Quigley | 10/12/10 | Updated, new terminology for Ehrlichia/Anaplasma, sample prep, and Misys order entry |
| 8 | Al Quigley | 06/01/11 | Updated, Reformatted (formerly Heme.B.07) |
| 9 | Al Quigley | 05/30/12 | Updated for preparation of thick smear |
| 10 | Al Quigley | 08/21/14 | Added codes for preliminary resulting. Changed order code from MAL to MALP. |
| 11 | Al Quigley | 03/28/16 | Added Hyperlink to PARQ procedure. |
| 12 | Al Quigley | 05/25/17 | Sysmex XN 3000 application. |
| 13 | Michele Koester | 3/5/2024 | Reformatted and revised to specific parasite identification. Changed coded result options |