TITLE: BLOOD PARASITE PREPARATION PROCEDURE

I. PURPOSE

The purpose of this document is to provide guidance on how to prepare and perform a smear for blood parasite identification.

II. PRINCIPLE

Positive diagnosis of malaria is demonstrated by malaria parasites (Plasmodium species) in stained blood films. This preparation is also used for the recovery and identification of other blood parasites such as African trypanosomes, babesia, Chagas disease (American trypanosomes), leishmania, malaria, and toxoplasma. In nature, many bloodborne parasites are spread by insects (vectors), so they are also referred to as vector-borne diseases. Toxoplasma gondii is not transmitted by an insect (vector). Whole blood is concentrated into cell layers and the top layer of RBCs is removed. This process selectively recovers the larger reticulocytes and helps eliminate platelets which may be confused with the parasites when superimposed on the red blood cells. The smears are made with the same thickness as peripheral blood smears.

III. SCOPE

This procedure will be used by the UR Medicine Labs, Strong Memorial Hospital, Hematology-Chemistry laboratory staff.

IV. RESPONSIBILITIES

TITLE	RESPONSIBILITY
Laboratory Director	Approval of Procedure
Supervisory Staff	Implementation of procedure
Staff	Adherence to procedure

V. SPECIMENS

A. EDTA blood specimen or 5cc of whole blood in a 50 ml flask with glass beads (blood must be fresh and collected during the patients chill or fever spike)

B. The LIS will automatically generate a blood parasite antigen test (MALAG) when a blood parasites smear (MALAR) is ordered. Similarly, when a MALAG is ordered, a MALAR test is added.

C. Positive results on MALAR and/or MALAG will be sent to NYS for final identification.

VI. QUALITY CONTROL

A. A positive and negative control for blood parasites should be run to check stain quality minimally with each new shipment or lot and once per month of use[G]. Since stain reagent lots are changed frequently, a positive and negative QC slide will be run with each blood parasite smear that is ordered.

B. To prepare quality control slides from a patient's blood:

1. 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.

2. Negative QC slide: Choose a previously tested patient blood sample that test negative for blood parasites.

3. Make as many thin smears as possible, preferably within one hour after the blood was drawn from the patient.

4. Allow the smears to dry quickly, using a fan or blower at room temperature.

5. Label appropriate as "Pos for BP" or "Neg for BP".

6. Fix the smears in absolute (100%) methanol; allow them to dry.

7. Place them, touching front to back, in a box without separating grooves.

8. Label the outside of the box with the contents, date and "Blood Parasite control slides."

9. Store at -70°C (or colder) until needed for use.

10. 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.

C. If aberrant quality control results are noted, patient results should not be reported.

D. 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.

E. Any major problems 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.

VII. SPECIAL SAFETY PRECAUTIONS

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.

VIII. MATERIALS

A. Equipment
   1. Centrifuge
   2. MIDAS stainer

B. Supplies
   1. Wintrobe Tubes
   2. 9" Pasteur Pipettes
   3. Watch glass
   4. Frosted end glass slides
   5. Slide pusher
   6. Positive and negative blood parasite control slides

C. Reagents
   1. Methanol
   2. Wright’s stain
   3. Phosphate Buffer
   4. Giemsa stain

IX. PROCEDURE – (STEP/ACTION)

A. If the flask with 5cc of whole blood is used, remove all traces of fibrin clots with several wooden applicator sticks.

B. Using the 9" Pasteur pipettes fill four (4) wintrobe tubes with the blood specimen and label with the patient's name, accession number and "MP".

C. Centrifuge at 2500 RPM for 10 minutes.

D. Remove the plasma from the wintrobe tubes with a 9" pasteur pipette. Save some of the plasma on the watch glass and discard the rest.

E. Remove the entire buffy coat layer from the tubes and discard along with the pipette.

F. Taking a clean pipette, remove the top layer of red cells and place on the watch glass. Rinse the pipette with some of the saved plasma and add to the red cells. Mix with the end of a Wintrobe tube.

G. Make four pushed smears. Label the slides with the patient's name, accession number, date and "MP". Stain two of the slides on the Midas stainer along with one positive and one negative blood parasite quality control smear.

H. Review smears for presence of blood parasites. At least two technologists should review each malarial prep slide. The feathered edge and the body of smear should be reviewed for microfilaria and trypanosomes under low power. At least 300 high power fields should be reviewed.

I. Positive Malaria or Babesia smears: Positive smears have the percent parasitemia or number of organisms/1000 RBCs determined using the Miller disc method. Count the red blood cells in square B (the small square) of the Miller disk while counting the red blood cells infected with the parasite in square A of the miller disk until 112 red cells from square B has been reached. See Appendix 1 for using the Miller disk. When counting the infected red cells, only count each red cell containing the parasite once even if it contains multiple parasites. Do not count extracellular parasites when determining the percent parasitemia. See Result Reporting section for more details.

J. Other possible parasites (microfilaria, trypanosomes, etc.): Note presence of parasite with description and send for pathology review.

K. Positive findings need to be reviewed by the Hematopathologist for further identification. The two unstained smears should be stained using Giemsa Plus Stain Kit (See SH.CP.AU.hem.0122 procedure).

L. Once the hematopathologist review is complete, subsequent slide preps on this patient do not need to have special staining performed. The two unstained smears can then be filed.

M. Notify a supervisor of any positive blood parasite findings.

X. LIMITATIONS

NA

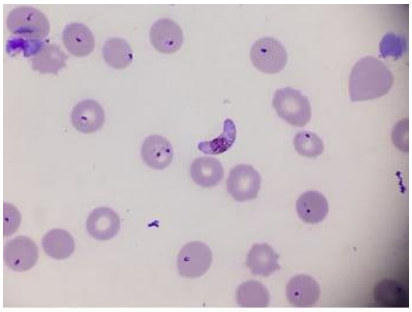
XI. CALCULATIONS

See Appendix 1 – Using the Miller Disk

XII. INTERPRETATION

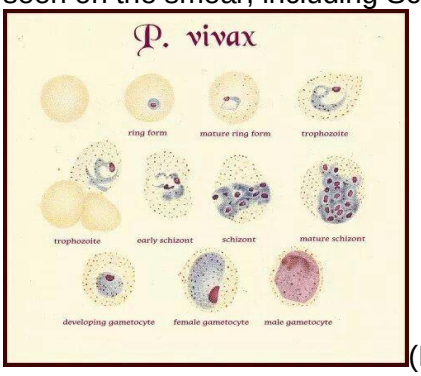
A. Four species of Plasmodium may be found in man:

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.



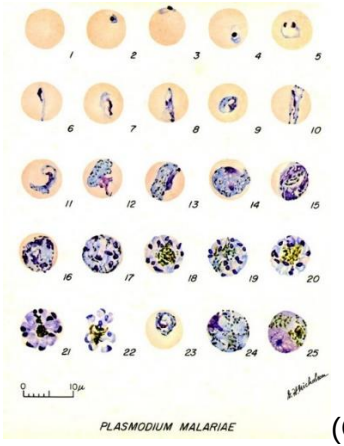
(E)
See also: https://www.cdc.gov/dpdx/malaria/

2. P. vivax: Chills and fever recur at 48-hour intervals. All forms may be seen on the smear, including Schuffner's Granules.



(D)
See also: https://www.cdc.gov/dpdx/malaria/

3. P. malariae: Chills and fever recur at 72-hour intervals. Round gametocytes or all six forms are seen.



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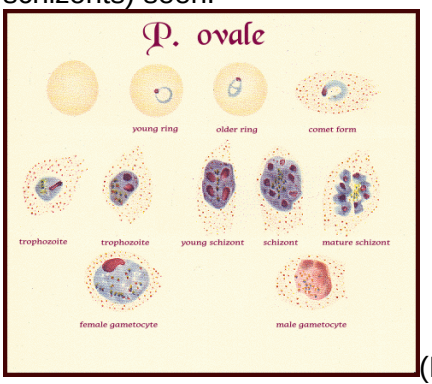
See also: https://www.cdc.gov/dpdx/malaria/

4. P. ovale: A very rare, benign form. Oval presegmented forms (early schizonts) seen.



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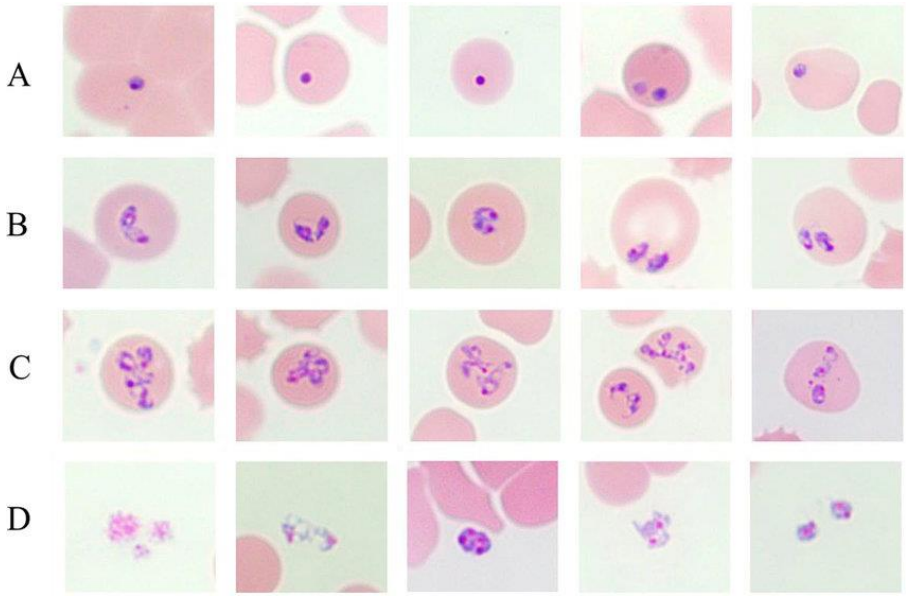
See also: https://www.cdc.gov/dpdx/malaria/

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.

For a comparison chart of Plasmodium species, see the link below:
https://www.cdc.gov/dpdx/resources/pdf/benchAids/malaria/Malaria_Comparison_p1-2.pdf

B. Babesia: *Babesia* parasites resemble *Plasmodium falciparum*, however *Babesia* has several distinguishing features: the parasites are pleomorphic (vary in shape and size), can be vacuolated, and do not produce pigment.



(F)

For more information on babesia/babesiosis, please go to the links below:
https://www.cdc.gov/dpdx/babesiosis/index.html
https://www.cdc.gov/dpdx/resources/pdf/benchAids/Babesia_benchaid.pdf

C. Microfilariae: Several species of microfilariae can be seen in the blood. For more details, please refer to Color Atlas of Hematology: An Illustrated Field Guide Based on Proficiency Testing, printed by CAP, 1998, pp. 276-279 or go to one of the links below:
https://www.cdc.gov/dpdx/mansonellosis/
https://www.cdc.gov/dpdx/resources/pdf/benchAids/Introduction_filariasis_who.pdf

D. Protozoan parasites (non-malarial): This group includes both African and American trypanosomes. For more details, please refer to Color Atlas of Hematology: An Illustrated Field Guide Based on Proficiency Testing, printed by CAP, 1998, pp. 280-283 or go to one of the links below:
https://www.cdc.gov/dpdx/trypanosomiasisafrican/
https://www.cdc.gov/dpdx/trypanosomiasisamerican/

E. It can be helpful to correlate the blood parasite smear (MALAR) results with malaria antigen results (MALAG).

XIII. RESULT REPORTING

A. Reference range: Negative

B. Blood parasite smears are reported as:

- "Negative" - no malaria or other blood parasites seen. Automatic comment will generate on report:

NEGATIVE FINDING IS NOT DIAGNOSTIC, REPEAT DRAW RECOMMENDED DURING FEBRILE PERIOD IF CLINICALLY INDICATED.

- "Suspicious" – No % parasitemia will be entered. Suspicious results will be changed to "positive" or "negative" based on supervisor and/or pathology review results.

- "Positive" - Positive findings need to be reviewed by the Hematopathologist for further identification. Final identification will be performed by NYSDOH, the results for which will be entered by the Microbiology department.

C. The % parasitemia test and the pathology review test (PATHR) will reflex with the entry of a positive or suspicious finding in the LIS. Refresh the template by pressing the "refresh" button or F5. The % parasitemia can then be entered.

D. Enter the result of the % parasitemia to the tenth's place (e.g. 1.2). DO NOT verify this result – press F12.

E. Positive findings are considered a critical value and are to be reported to the ordering physician immediately, with the understanding that the finding is preliminary and needs to be confirmed by a pathologist.

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. Clinical Diagnosis by Laboratory Methods, Todd & Sanford, 14th edition, p. 978

B. Clinical Hematology, Wintrobe, 6th edition, pp. 616-617.

C. Coatney, G. R., W. E. Collins, M. Warren, and P. G. Contacos. 1971. The primate malarias. U. S. Government Printing Office, Washington, D.C.

D. Pinterest: rph.wa.gov.au (P. ovale and P. vivax images)

E. https://www.shutterstock.com/search/plasmodium+falciparum

F. https://www.researchgate.net/figure/Babesia-spp-in-a-thin-blood-smear-stained-with-5-Giemsa-on-May-30-2017-from-a-patient_fig1_333683850

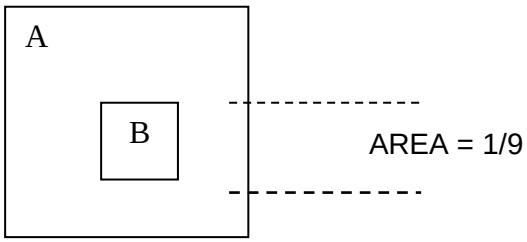
G. New York State Department of Health, Clinical Laboratory Standards of Practice, Part 2 – Specialty requirements, June 2017, p.134.

H. https://www.cdc.gov/dpdx.

I. https://www.cdc.gov/dpdx/diagnosticprocedures/blood/staining.html

APPENDIX 1 MILLER DISK METHOD FOR COUNTING

The Miller Disk method for counting is a method that employs the aid of an optical device. The disk is a reticle that is placed in one ocular of the microscope. Engraved on the disk is a small square B surrounded by a large square A, the area of which is nine times that of the small square.



By enumerating the red cells in the square B while counting the reticulocytes or red cells infected with malaria in the entire area of square A (including those inside square B), the percentage of reticulocytes or the percent parasitemia can be determined. Thus, when a total of 112 red cells have been counted in the small square B, the actual percentage of reticulocytes is based on 1000 red blood cells.

EQUIPMENT

Miller Disk - (Vendor: Reichert Scientific Instruments)
Microscope with oil immersion objective
Counting device

METHOD

Using the oil immersion objective, focus on the slide the count is to be performed on. Count the number of red blood cells in square B. DO NOT count any red blood cells which touch the lines of square B. Any retic or rbc containing malaria within square B is counted as a RBC. Count the number of reticulocytes or rbcs containing malaria in all of square A. Retics or cells containing malaria touching the lines of square A are NOT counted. Retics or cells containing malaria touching the lines of square B are to be counted. Continue counting adjacent fields until you have counted a total of 112 red blood cells.