Preparing Blood Films for Malaria and Other Blood Parasites – Medical Centers

Table of Contents

Preparing Blood Films for Malaria and Other Blood Parasites – Medical Centers, Continued

Purpose

This procedure provides instructions on how to collect, prepare, and transport blood film slides for malaria and other blood parasites. The slides are used in conjunction with the BinaxNOW® Malaria Test.

Scope

The intended users of this document include Phlebotomists, Clinical Laboratory Scientists (CLS), and Managers.

Policy

- The area Medical Center laboratory is responsible for collecting and preparing blood films for malaria and other blood parasites prior to transporting to the Regional Reference Laboratories (RRL).
- The RRL is responsible for reading and reporting the results on blood/buffy coat films for malaria and other blood parasites prior to transporting the slides to the Public Health Department of the county of origin.
- Blood smears must be prepared in conjunction with the BinaxNOW® Malaria Test on all patients tested, regardless of the BinaxNOW® Malaria Test result.

Specimen Source and Collection

The optimum time for taking blood for parasite examination varies with the particular parasite suspected. Single collections may not reveal organisms; successive blood films should be taken every six to eight hours for up to three days. Samples must be taken before any antimalarial drugs are used. The following are examples of the optimum collection times for specific parasites:

Condition/Parasite Suspected	Optimum Collection Time
Malaria (*Plasmodium* species)	Immediately upon suspicion of malaria; if negative, midway between chills every 6-8 hours for up to 3 days
Babesiosis (*Babesia* species)	Immediately upon suspicion; if negative, every 6-8 hours for up to 3 days (organisms may be found at any time of day)
Chagas' Disease (*T. cruzi*)	First month of infection and in subsequent febrile periods; buffy coat concentration is recommended
African trypanosomiasis (*T. brucei rhodesiense/gambiense)*	Acute phase of infection; after several months to a year, organisms are better demonstrated in CSF and lymph node aspirate material than in blood
Filariasis (*Wuchereria bancrofti)*	For filaria with nocturnal periodicity, collect at night around midnight; for filaria with diurnal periodicity, collect at noon

Continued on next page

Preparing Blood Films for Malaria and Other Blood Parasites – Medical Centers, Continued

Specimen Storage

- Blood collected by finger puncture must be used immediately.
- Blood collected by venipuncture must be stored at ambient temperature and used within one hour after collection.

Specimen Rejection

Any of the following conditions will lead to cancelation of the test: Source other than whole blood, clotted blood, unlabeled slides, patient name/ID discrepancy.

Materials and Supplies

The following materials and supplies are needed:

- Finger puncture and/or venipuncture supplies, including EDTA capillary tubes, and/or EDTA Vacutainer tubes
- 25 x 75 mm glass microscope slides, with frosted end, free of grease, lint, scratches, chips or fingerprints (clean with 70% alcohol prior to use)
- Absorbent towels/gauze
- Plastic pipette
- 70% Alcohol (store in flammable storage cabinet or container)
- Biohazardous waste container

Safety Precautions

Observe standard precautions when collecting blood. Follow blood collection protocols and procedures. Wear personal protective equipment, as required. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens. Refer to the safety manual for additional information.

Quality Control

Blood films stained with Giemsa remains the gold standard for diagnosing blood parasites. Species are life cycle stages that can be readily seen, and the amount of parasitemia is quantifiable. **Both thin and thick blood films must be used for morphological detection of malarial parasites**, and are prepared from a finger puncture or a peripheral venipuncture. Thick films allow a larger amount of blood to be examined, thus increasing the possibility of detecting light parasitemia; thin films allow the identification of parasite and determination of the degree of parasitemia.

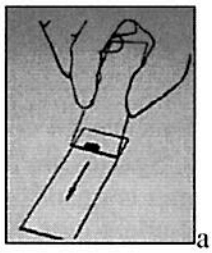
Buffy coat film slides contain concentrates of white blood cells, monocytes, and platelets. These slides are useful for detection of amastigotes in visceral leishmaniasis, as well as for diagnosing trypanosomiasis and filaraiasis. In the event a request related to one of these diseases is received, please refer to the instructions in Appendix 1: Buffy Coat Film Preparation for Leishmania and Other Blood Parasites.

Continued on next page

Preparing Blood Films for Malaria and Other Blood Parasites – Medical Centers, Continued

Blood Collection, Finger Puncture

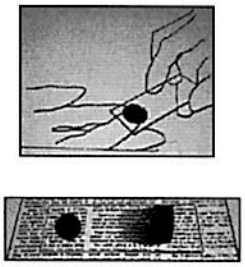
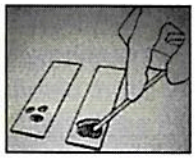
Follow the steps below when collecting blood by finger puncture.

Step	Action
1	Using gauze squares soaked in 70% alcohol, clean the palmar surface of the fingertip. Allow to air dry prior to the puncture.
2	Prick the finger with a sterile, non reusable lancet deep enough to obtain sufficient free-flowing blood and prepare **2 thin and 2 thick blood smears.** *Note:* ***Care must be taken to not squeeze the finger, as tissue juice in the sample can potentially cause lowered probability of detection in low parasitemia samples.***
3	**Prepare 2 thin blood films** on pre-cleaned glass slides: • Place a drop of blood near the frosted end of a glass microscope slide. • Hold a second microscope slide (spreader) with a polished edge at a 40-45° angle and draw into the drop of blood. Allow the blood to spread up to whole width of the spreader slide. • Push the spreader slide rapidly and smoothly to the opposite end of the slide, pulling the blood behind it. **Figure 1**  a  *Note:* ***A well prepared thin film is thick at one end and thin at the other end. The thin end should be at least 2 cm long and will show one layer of evenly distributed RBCs with no cell overlap.***

Continued on next page

Preparing Blood Films for Malaria and Other Blood Parasites – Medical Centers, Continued

Blood Collection, Finger Puncture, continued

Step	Action
4	**Prepare 2 thick films by contact method.** • Touch the slide to the drop of rounded up blood on the finger. • Rotate the slide to form a circular film the size of dime or nickel and just thick enough so that newspaper print can be barely read through it. **Figure 2**  **- OR -** **Prepare the thick film by puddle method.** • Place 2-3 drops of blood on a slide to form a thick film the size of a dime or nickel. • Using an applicator stick or corner of another slide, stir the film for 30 seconds to remove fibrin strands. **Figure 3**  ***Alert!*** ***This technique can ONLY be used with blood containing no anticoagulant.***

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Preparing Blood Films for Malaria and Other Blood Parasites – Medical Centers, Continued

Blood Collection, Finger Puncture, continued

Step	Action
5	Apply pressure to the puncture site after the collection of specimen with gauze or cotton until bleeding stops. Then apply a bandage.
6	Label slides with the required patient information: **Last name**, **Accession number**, and **Time of collection**.
7	• Dry blood films in a **flat, horizontal position** at room temperature or in a 25°C incubator. • Thick films take 8-12 hours to dry thoroughly. The use of a fan may hasten the drying time to 1-4 hours. • Alternatively, thick smears can be dried in a 37°C incubator for 10-15 minutes* without fixation of the RBCs. **** Warning!*** ***Do NOT incubate beyond 15 minutes using this method.***
8	After drying, the blood films are ready to be transported to the RRL for staining and reading.

Blood Collection, Venipuncture

Follow the steps below when collecting blood by venipuncture.

For specific instructions for making buffy coat slides for *Leishmania* and other blood parasites, refer to the instructions in Appendix 1.

Step	Action
1	Perform venipuncture procedure.
2	• Prepare the blood films at the time of phlebotomy from the blood remaining in the needle (before mixing with the anticoagulant) or from blood in anticoagulant (EDTA) **within one hour of collection***. **** Warning!*** ***Delays in smear preparation may cause morphological changes in the parasite, inability to demonstrate Schuffner's stippling, and the possibility of thick film washing off during the staining procedure.*** • When preparing blood films using blood in anticoagulant, open the tube in a Biosafety cabinet or use a face shield.

Continued on next page

Preparing Blood Films for Malaria and Other Blood Parasites – Medical Centers, Continued

Blood Collection, Venipuncture, continued

Step	Action
3	Prepare 2 thin blood films (from the blood remaining in the needle or well mixed EDTA blood): • Place a drop of blood near the frosted end of a pre-cleaned glass microscope slide (refer to **Figure 1** on Page 4 of this procedure). • Hold a second microscope slide (spreader) with polished edge at a 40-45° angle and draw into the drop of blood. Allow the blood to spread up to whole width of the spreader slide. • Push the spreader slide rapidly and smoothly to the opposite end of the slide, pulling the blood behind it. *Note:* ***A well-prepared thin film is thick at one end and thin at the other end. The thin end should be at least 2 cm long and will show one layer of evenly distributed RBCs with no cell overlap.***
4	Prepare 2 thick films (from the blood remaining in the needle or from the well mixed EDTA blood). • Place a drop of blood the size of a dime or nickel on the slide. • Rotate the slide to form a circular film and just thick enough so that newspaper print can be barely read through it (refer to **Figures 2 and 3** on Page 5 of this procedure).
5	Also, the thick film may be prepared by the **puddle method** (from the blood remaining in the needle only). ***Warning! This technique can only be used with blood containing NO anticoagulant.*** • Place 2-3 drops of blood on a slide to form a thick film the size of a dime or nickel. • Using an applicator stick or corner of another slide, stir the film for 30 seconds to remove fibrin strands.
6	Label slides with the required patient information: **Last name**, **Accession number**, and **Time of collection**.
7	• Dry blood films in a **flat, horizontal position** at room temperature or in a 25°C incubator. • Thick films take 8-12 hours to dry thoroughly. The use of a fan may hasten the drying time to 1-4 hours. • Alternatively, thick smears can be dried in a 37°C incubator for 10-15 minutes* without fixation of the RBCs. ****Warning! Do NOT incubate beyond 15 minutes using this method.***

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Preparing Blood Films for Malaria and Other Blood Parasites – Medical Centers, Continued

Next Steps

Step	Action
1	Ensure slides are mostly dry. The thick films can be in the process of drying, but do not send dripping wet. Place in a container similar to the one shown in **Figure 5**. Slides should not be touching each other.
2	Send the 2 thick and 2 thin smears to the **RRL Bacteriology Department** on the next regularly scheduled Courier run.

Figure 5

Non-Controlled Documents

The following non-controlled documents support this procedure:

- Garcia, L.S. and Bruckner, D., Diagnostic Medical Parasitology, Fifth Edition, 2007. ASM, Washington, DC.
- Garcia, L.S., Essential Procedures for Clinical Microbiology, 2007, ASM, Washington, D.C.
- *Laboratory Diagnosis of Blood-borne Parasitic Diseases: Approved Guideline*. CLSI Document M15-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2000.
- Laboratory Procedures for Diagnosis of Blood-Borne Parasitic Diseases (Cumitech 46, 2008); ASM Press, Washington, D.C.

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Preparing Blood Films for Malaria and Other Blood Parasites – Medical Centers, Continued

Controlled Documents

The following controlled documents support this procedure.

Procedure	Number
Transferring-Tracking Specimens	LIS.SCPMG.004
Canceling Test Orders	LIS.SCPMG.032
Generating PathNet Microbiology Management Reports	LIS.SCPMG.013
Reference	**Number**
Appendix 1: Buffy Coat Film Preparation for *Leishmania* and Other Blood Parasites – Medical Centers	MICRO.SCPMG.004

Author(s)

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Preparing Blood Films for Malaria and Other Blood Parasites – Medical Centers

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Preparing Blood Films for Malaria and Other Blood Parasites – Medical Centers

HISTORY PAGE

Type of Change: New Major, Minor	Description of Change(s)	Director of Microbiology Review/Date	Operations Director, Area Laboratory Review/Date	CLIA Laboratory Director Review/Date	Date Change Implemented
New					

Appendix 1: Buffy Coat Film Preparation for *Leishmania* and Other Blood Parasites – Medical Centers

Purpose

This document provides instructions on how to prepare a buffy coat film from venous blood for detection of *Leishmania* and other blood parasites. A buffy coat film is the result of a concentration procedure designed to increase the number of organisms recovered from blood specimens when leishmaniasis, trypanosomiasis, or filariasis is suspected. The sensitivity of the buffy coat film is much greater than that of a routine thick film.

Specimen Storage

Blood collected by venipuncture must be stored at ambient temperature and used within one hour after collection.

Materials and Supplies

The following materials and supplies are needed:

- Venipuncture supplies, including EDTA Vacutainer tubes
- 25 x 75 mm glass microscope slides, with frosted end, free of grease, lint, scratches, chips or fingerprints (clean with 70% alcohol prior to use)
- Absorbent towels/gauze
- Plastic pipette
- Centrifuge
- 70% Alcohol (store in flammable storage cabinet or container)
- Biohazard waste container

Safety Precautions

Observe standard precautions when collecting blood. Follow blood collection protocols and procedures. Wear personal protective equipment, as required. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens. Refer to the safety manual for additional information.

Preparing a Buffy Coat Film

Perform venipuncture procedure. Follow the steps on Page 2 of this procedure to prepare a buffy coat film using whole blood in EDTA anticoagulant. The buffy coat contains concentrates of white blood cells, and is useful for detection of amastigotes in visceral leishmaniasis, as well as in diagnosing trypanosomiasis and filariasis (refer to Figure 1 on Page 2 of this procedure to view the location of the buffy coat layer in a tube of centrifuged blood).

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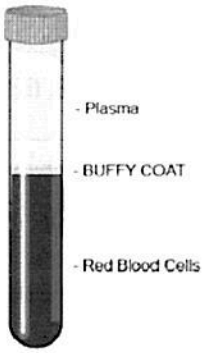
Appendix 1: Buffy Coat Film Preparation for *Leishmania* and Other Blood Parasites – Medical Centers, Continued

Preparing a Buffy Coat Film, continued

Figure 1



Step	Action
1	Centrifuge the tube of blood for 15 minutes at 3000xg.
2	• Open the tube in a Biosafety cabinet or use a face shield. • Using a pipette, remove and discard the plasma into a biohazard waste container.
3	Using a fresh pipette, prepare 4 thin smears: • Place a drop of the buffy coat near the frosted end of a glass microscope slide. • Hold a second microscope slide (spreader) with polished edge at a 40-45° angle and draw into the drop of buffy coat. Allow the buffy coat to spread up to whole width of the spreader slide. • Push the spreader slide rapidly and smoothly to the opposite end of the slide, pulling the buffy coat behind it. • Discard the used pipette in the biohazard waste container.
4	Label slides with the required patient information: **Last name**, **Accession number**, and **Time of collection**.
5	Dry buffy coat films in a **flat, horizontal position** at room temperature or in a 25°C incubator*. ** Warning! Do NOT apply heat.*

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Appendix 1: Buffy Coat Film Preparation for *Leishmania* and Other Blood Parasites – Medical Centers, Continued

Next Steps

Step	Action
1	Ensure slides are mostly dry. Place in a container similar to the one shown in **Figure 2**. Slides should not be touching each other.
2	Send the 4 smears to the **RRL Bacteriology Department** on the next regularly scheduled Courier run.

Figure 2

Non-Controlled Documents

The following non-controlled documents support this reference:

- Garcia, L.S. and Bruckner, D., Diagnostic Medical Parasitology, Fifth Edition, 2007. ASM, Washington, DC.
- Garcia, L.S., Essential Procedures for Clinical Microbiology, 2007, ASM, Washington, D.C.
- *Laboratory Diagnosis of Blood-borne Parasitic Diseases: Approved Guideline*. CLSI Document M15-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2000.
- Laboratory Procedures for Diagnosis of Blood-Borne Parasitic Diseases (Cumitech 46, 2008); ASM Press, Washington, D.C.

Author(s)

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Appendix 1: Buffy Coat Film Preparation for *Leishmania* and Other Blood Parasites – Medical Centers

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Appendix 1: Buffy Coat Film Preparation for *Leishmania* and Other Blood Parasites – Medical Centers

HISTORY PAGE

Type of Change: New Major, Minor	Description of Change(s)	Director of Microbiology Review/Date	Operations Director, Area Laboratory Review/Date	CLIA Laboratory Director Review/Date	Date Change Implemented
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