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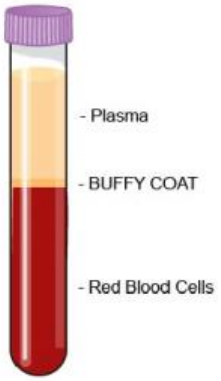
Appendix 1: Buffy Coat Film Preparation for *Leishmania* and Other Blood Parasites – Medical Centers, Continued

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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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.

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Reviewed and approved by:

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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					