

## Appendix 1: Buffy Coat Preparation for Leishmania and other Blood Parasites

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**Purpose** To provide instructions on how to prepare a buffy coat for Leishmania in the event it is requested by a physician. The buffy coat is a concentration procedure designed to increase the number of organisms recovered from blood specimens when trypanosomiasis, filariasis, and Leishmaniasis is suspected. The sensitivity of the buffy coat is much greater than that of a routine thick film.

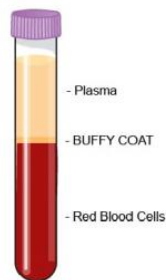
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**Supplies needed** The following supplies are needed:

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

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**Buffy coat preparation** Follow the procedure below 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. See Figure 1 below.



**Fig. 1**

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## Appendix 1: Buffy Coat Preparation for Leishmania, continued

**Buffy coat preparation, cont'd**

Continue to follow the procedure below to prepare a buffy coat film..

Step	Action
1	Centrifuge the tube of blood for 15 minutes at 3000xg.
2	Using a pipette, remove and discard the plasma into a biohazard waste container.
3	Using a fresh pipette, prepare 4 thin smears: <ul style="list-style-type: none"> <li>• Place a drop of the buffy coat near the frosted end of a glass microscope slide.</li> <li>• Hold a second microscope slide (spreader) with polished edge at a 40-45° angle and draw into the drop of blood. Allow the buffy coat to spread up to whole width of the spreader slide.</li> <li>• Push the spreader slide rapidly and smoothly to the opposite end of the slide, pulling the blood behind it.</li> <li>• Discard the used pipette in the biohazard waste container.</li> </ul>
4	Label slides with the required patient information: Last name, Cerner accession number, and time of collection.
5	Dry buffy coat films in a <b>flat, horizontal position</b> at room temperature or 25°C incubator. <b>No heat should be applied.</b>

**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.



Fig 2

*Continued on next page*

**References**

The following references are used in 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. NCCLS M15-A. Volume 20, Number 12.
- Laboratory Procedures for Diagnosis of Blood-Borne Parasitic Diseases (Cumitech 46, 2008); ASM Press, Washington, D.C.

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