CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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M15-A

Laboratory Diagnosis of Blood-borne Parasitic Diseases; Approved Guideline

This document provides guidance on specimen collection, optimum timing for preparing blood films, blood film preparations, staining procedures, examination of specimens, and identification of parasites.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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Laboratory Diagnosis of Blood-borne Parasitic Diseases; Approved Guideline

Abstract

CLSI document M15-A—*Laboratory Diagnosis of Blood-borne Parasitic Diseases; Approved Guideline*, presents instructions for preparation of thick and thin blood films, the appropriate use of stains, and methods to assist in the diagnosis of many parasitic diseases. Procedures for blood collection by skin puncture and venipuncture, techniques for preparing films for blood parasite examination, and steps for preparing Giemsa stain and other reagents, including a special stain for microfilariae, are provided. The optimum times for preparing blood films for five particular parasites—*Plasmodium* species (malaria), *Babesia* species, *Trypanosoma cruzi* (Chagas' disease), African trypanosomiasis, and filariasis—are identified and explained. A thorough list of blood film examination supplies is included. Basic guidelines and reference materials for the identification of blood parasites are given.

Other than babesiosis, vector-transmitted, blood-borne parasitic diseases are not endemic to temperate climates. However, laboratories may be called upon to detect and identify an etiological agent in suspected infections. This document is, therefore, useful for the performance of such laboratory procedures.

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Foreword

Although vector-transmitted, blood-borne parasitic diseases other than babesiosis are not endemic to temperate climates, laboratories may be required to detect and identify an etiologic agent in suspected infections. While epidemiologic, clinical, immunodiagnostic, and molecular techniques are available for diagnosis of some diseases, laboratory diagnosis is still based on morphological identification of the etiologic agent for most diseases. Films prepared from capillary or venous blood are of primary importance in diagnosing malaria, babesiosis, the acute stages of Chagas' disease, African trypanosomiasis, and filariasis (except *Onchocerca volvulus* and *Mansonella streptocerca*, in which skin snips rather than blood films are usually examined). The ability to prepare proper thick and thin blood films, to use the appropriate stains, and to detect and identify parasites is extremely important.

Some of the procedures used in this guideline have been adapted and incorporated from NCCLS approved standard H4—*Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture*, which was prepared by the NCCLS Subcommittee on Blood Collection Procedures.

Much of the other material presented was adapted from the Centers for Disease Control and Prevention training manual for the course "Laboratory Diagnosis of Blood Parasites" when this project was begun under the direction of the first NCCLS parasitology subcommittee, chaired by Dr. George R. Healy.

In preparing this approved guideline, the subcommittee carefully considered comments received during consensus review of the proposed guideline. A summary of the comments and committee responses is included in this document.

Standard Precautions

Because it is often impossible to know what might be infectious, all human blood specimens are to be treated as infectious and handled according to "standard precautions." Standard precautions are new guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals.* Infection Control and Hospital Epidemiology. CDC. 1996; Vol. 17: 1: 53-80.), [MMWR 1987;36(suppl 2S):2S-18S] and (MMWR 1988; 37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure, refer to NCCLS document M29—*Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*.

Key Words

Blood films, etiologic agents, malaria, parasites

Laboratory Diagnosis of Blood-borne Parasitic Diseases; Approved Guideline

1 Introduction

Preparation of thick and thin blood films, the appropriate use of stains, and detection and identification of parasites are crucial to clinical diagnosis of many parasitic diseases. This document presents guidelines on specimen collection, blood film preparation, staining methods, examination, and identification procedures. Recommendations for the optimum timing of specimen collection to coincide with parasite activity cycles are also included.

2 Optimum Time for Preparing Blood Films for Parasite Identification

The optimum time for taking blood for parasitologic examinations varies with the particular parasite suspected.

2.1 Malaria (*Plasmodium* species)

Although the optimum time is about midway between chills to ensure obtaining stages on which species identifications can be made, BLOOD COLLECTION SHOULD BE PERFORMED IMMEDIATELY UPON FIRST SUSPICION OF MALARIA. Furthermore, since single films may not reveal organisms, successive films every six to eight hours for up to three days are sometimes necessary. Blood samples must be taken before any antimalarial drugs are used to ensure demonstration of organisms if the patient does have malaria.

For practical purposes, AT THE TIME OF ADMISSION OF THE PATIENT, at least four blood films—two thin and two thick (one slightly thinner than usual, one regular)—are prepared.

MALARIA IS CONSIDERED TO BE ONE OF THE FEW PARASITIC INFECTIONS THAT CAN BE IMMEDIATELY LIFE THREATENING. ANY LABORATORY PROVIDING THE EXPERTISE TO IDENTIFY MALARIAL PARASITES SHOULD DO SO ON A 24-HOUR BASIS, SEVEN DAYS A WEEK.

Handling STAT Malaria Requests in the Clinical Laboratory

(1) Prepare at least three thick and three thin blood films as soon as possible after EDTA blood is collected.
(2) Use a fan to dry the smears (or place over the grid in the front of a laminar flow biological safety cabinet).
(3) Dip the thickest end of one thin film briefly in water to lyse the RBCs; blow dry.
(4) Stain this thin film quickly by the method used in the laboratory for hematology differential smears.
(5) Examine this thin film, using the lysed thick area as a "pseudo" thick film. Call the Stat result to the requesting physician or ward. (Parasites should be visible with Wright's stain or Wright-Giemsa, but for definitive diagnosis, use Giemsa.)
(6) Dip the feathered end of a second thin film briefly into absolute methanol to fix the RBCs.
(7) Prepare 2.5 % working Giemsa stain. Stain the second thin film and one unstained thick film for 45 minutes. Rinse in buffered water, dry, and examine.
(8) Retain any extra blood films and EDTA blood to send to a reference lab if needed.

NOTE: Steps (6) and (7) may be postponed until day shift if necessary.

2.2 Babesiosis (*Babesia* species)

Although diagnostic methods for babesiosis are similar to malaria, organisms may be found at any time of day. As in suspected cases of malaria, blood films should be prepared at the time the patient is admitted and, if no parasites are detected, successive films every six to eight hours for up to three days are sometimes necessary.

2.3 Chagas' Disease (*Trypanosoma cruzi)*

Trypomastigotes are generally seen in circulating blood during the early acute phase (first month of infection) and in subsequent febrile periods. Films prepared at other times are of little diagnostic value.

In addition to thick and thin blood films, a buffy coat concentration is recommended. The blood may be obtained by venipuncture using a tube containing EDTA anticoagulant or by skin-puncture using a glass or plastic tube containing EDTA anticoagulant, with a capacity of 200 to 500 µL. The blood should be mixed well by gently inverting the capped tube 8 to 10 times, then centrifuged at 500 g for 10 minutes. Using a Pasteur pipet, transfer a portion of the red blood cells just below the buffy coat to glass slides for subsequent staining.[1] See Section 6.3.

2.4 African Trypanosomiasis

Organisms are present in blood during the acute phase of infection. After several months to a year, trypomastigotes are better demonstrated in spinal fluid than in blood. Stained cytospin preparations of cerebrospinal fluid may also be helpful in suspected cases. Lymph node aspirate materials can also be stained with Giemsa stain for the presence of trypomastigotes.

Concentration techniques as described in Section 2.3 can also be used for the recovery of the African trypanosomes.

2.5 Filariasis

Microfilariae of certain species of filariae show nocturnal or diurnal periodicity, and films for diagnosis should be made at the appropriate time. For nocturnal species (*Wuchereria bancrofti* and *Brugia malayi*) films are prepared at night, usually around midnight. For diurnal *Loa loa*, films are prepared around noon. This assumes the patient has been in the present time zone for a while (parasites have adjusted to the patient's present 24-hour activity cycle).

It may be necessary to draw blood at “off” hours to coincide with the appropriate periodicity for patients who have just returned from endemic areas (prior to readjustment to the local time zone). The other species of microfilariae are considered nonperiodic, and films may be made at any hour.

Microfilaremias (unless in nationals in endemic areas) are lower in most Americans and Europeans who have become infected, and simple thin and/or thick films may not allow detection of organisms. There are "concentration" procedures such as Knott's, citrate-saponin-acid, and the membrane filter method[2-6] that use larger quantities of blood in which organisms are more apt to be demonstrated.

Related NCCLS Publications*

GP2-A2 **Clinical Laboratory Procedure Manuals—Third Edition; Approved Guideline (1992).** This document describes the design, preparation, maintenance, and use of technical procedure manuals in the clinical laboratory.

H3-A4 **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Fourth Edition (1998).** This document provides methods for the collection of blood specimens by venipuncture and appropriate training program aimed at increasing analyte integrity and minimizing laboratory error. Includes a 25-step protocol for specimen collection, recommendations for "order of draw" and considerations for performing venipuncture on children.

H4-A4 **Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture; Approved Standard—Fourth Edition (1999).** The document provides proper collection techniques, as well as hazards to patients due to inappropriate specimen collection by skin puncture procedures.

M29-A **Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline (1997).** This document provides guidance on the risk of transmission of hepatitis B virus and human immunodeficiency viruses in the laboratory; specific precautions for preventing transmission of blood-borne infection during clinical and anatomical laboratory procedures.

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

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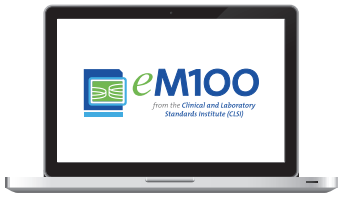

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