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M28-A2

Procedures for the Recovery and Identification of Parasites From the Intestinal Tract; Approved Guideline—Second Edition

This guideline addresses the collection, processing, and examination of intestinal tract specimens for the identification of parasites.

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

The diagnosis of parasites from the intestinal tract depends on the recovery and identification of the etiologic agents. Therefore, the ability to collect, process, and examine fecal specimens is important in terms of clinical relevance and patient care. Parasitic infections are not normally treated without demonstration of the specific causative agent. Thus, the ability to recover and identify these organisms is an important part of the overall microbiological responsibilities of the diagnostic laboratory.1,2

Communication of instructions to the patient, specimen collection and handling techniques, diagnostic tests, and result reporting are key components in proper patient management. Major sections of this document cover these topics, as well as equipment, reagents, and specific techniques used in diagnosing intestinal parasitic infections.

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Foreword

Although it is common to think of parasitic diseases as occurring only in the tropical areas of the world, many of the infections seen in the intestinal tract are endemic within the more temperate regions of the world, including the United States. In addition to these more common organisms, laboratories are also required to identify some of the less common intestinal parasites seen in travelers and proficiency testing specimens. The diagnosis of parasites from the intestinal tract depends on the recovery and identification of the etiologic agents. The ability to collect, process, and examine specimens from this body site is important in terms of clinical relevance and patient care. Parasitic infections are not normally treated without demonstration of the specific causative agent. Thus, the ability to recover and identify these organisms is an important part of the overall microbiological responsibilities of the diagnostic laboratory.

The Subcommittee on Parasitology, as part of the Area Committee on Microbiology, identified the need for a guideline for the examination of fecal specimens. The subcommittee also identified this topic as one that would apply to most laboratories providing diagnostic procedures in microbiology, specifically parasitology. This aspect of diagnostic parasitology often represents the majority of specimens submitted by both in- and out-patients. Even in a relatively small laboratory, the submission of fecal specimens for examination for parasites may occasionally occur.

Communication of instructions to the patient, specimen collection and handling techniques, diagnostic tests, and result reporting are key components in proper patient management. Major sections of this document cover these topics, as well as equipment, reagents, and specific techniques used in diagnosing intestinal parasitic infections.\textsuperscript{1-8}

The CLSI Working Group on Recovery/Identification of Parasites From the Intestinal Tract has revised the document to the second edition of the approved guideline. Last published in December of 1997, the document now includes the following enhancements:

\begin{itemize}
  \item Newer technologies have been addressed. Diagnostic kits that detect specific organism antigens are discussed.
  \item Appropriate definitions are now included.
  \item Additional modified acid-fast staining techniques for the identification of intestinal coccidia have been added to the document.
  \item Additional modified trichrome staining methods for the identification of intestinal microsporidia are now included.
  \item The section on fecal immunoassays and gene probes has been greatly expanded to include the newer diagnostic options.
  \item Additional information on chemofluorescent agents has been added.
  \item Specific ordering options, including the routine O&P examination and fecal immunoassays, have been included to serve as guidelines for clinician ordering recommendations.
\end{itemize}

Comments submitted on the first edition of the approved M28 document are addressed in an appendix in this publication. The working group urges the reader to send constructive suggestions for improving this document to CLSI so that we can evaluate the practical usefulness of the document to members of the healthcare community. We look forward to receiving comments and to the reader’s active participation in the CLSI consensus process.
Key Words

Diagnostic procedures, etiologic agents, intestinal tract, parasites
Procedures for the Recovery and Identification of Parasites From the Intestinal Tract; Approved Guideline—Second Edition

1 Scope

This guideline is intended to provide the readers with standardized procedures used for the recovery and identification of parasites from the intestinal tract. The intended audience includes those on the healthcare team, including laboratorians, microbiologists, parasitologists, physicians, public health personnel, and those in academic settings who are involved in teaching diagnostic medical parasitology.

The document is not intended to provide didactic training related to human parasite life cycles, organism morphology, clinical disease, pathogenesis, treatment, or epidemiology and prevention. However, the procedures provided are very comprehensive and discuss in detail the actual method, procedure notes and limitations, and information related to quality control and reporting of results.

2 Equipment for Fecal Specimen Examination

2.1 Microscope

High-quality microscopes with good resolving power are mandatory for the examination of specimens for parasites. Identification of the majority of organisms depends on morphologic differences, most of which should be seen using dissecting or regular microscopes.

2.1.1 Dissecting

A dissecting microscope should be available for examination of larger specimens (arthropods, some helminths, and various artifacts). The total magnification usually ranges from 10x to 45x. Some of the microscopes have a zoom capacity from 10x to 45x and others have fixed objectives (0.66x, 1.3x, and 3x) that can be used with 5x or 10x oculars. It is helpful to be able to use a light source either from under the specimen or directed onto the top of the specimen.

2.1.2 Brightfield

A binocular, brightfield microscope, with a minimum of 10x, 40x, and 100x (oil immersion) objectives and 10x oculars, should be available for use. Some laboratories also use a 4x objective. In addition to the above objectives, some laboratorians find the 40x, 50x, or 60x oil immersion lenses helpful, particularly for screening stained smears. Although 10x oculars are most commonly used, 12.5x and 5x are also available, but the smaller magnification of the 5x oculars may make final organism identification more difficult. Preferably, the microscope should have a built-in lamp, an adjustable substage condenser with an iris diaphragm, and a blue daylight filter. In the event that an adjustable condenser is not available, a fixed condenser is acceptable. The numerical aperture of the condenser should match the highest numerical aperture of the objective lenses (usually the lens with the highest magnification).

2.1.3 Fluorescence

For completing direct fluorescent antibodies (DFAs) for *Giardia/Cryptosporidium*, fluorescence for microsporidia or autofluorescence for *Cyclospora*, a fluorescent microscope with FITC, Calcofluor, and a blue filter set are also necessary.
2.1.4 Care of the Microscope

Microscopes should be covered when not in use, and all lenses should be cared for with lens paper only. Remember to use several layers of lens paper when cleaning the objective; one layer is insufficient for total oil removal. It is particularly important to remove all oil when work is finished. Avoid the use of xylene for cleaning optical surfaces. Follow the manufacturer’s guidebook when making any adjustments or changes.

2.2 Calibration (Ocular Micrometer)\(^1\)

One of the most important factors in the identification of parasites is size. It is essential that any laboratory performing procedures for the recovery and identification of parasites have a calibrated ocular micrometer available. Measurements are performed using a micrometer disk (usually calibrated as a line divided into 50 units) that is placed in the ocular of the microscope. Depending on the objective magnification used, these unit divisions will represent different measurements. Therefore, the ocular disk should be compared with a known calibrated scale, usually a stage micrometer with a scale of 0.1- and 0.01-mm divisions. After each microscope objective has been calibrated, the ocular containing the disk and/or the objectives cannot be interchanged with oculars and/or objectives from another microscope. Each microscope should be calibrated as a total package; the original oculars and objectives used to calibrate the microscope should be used when measuring organisms. Some laboratories use a separate ocular (containing the micrometer disk), which has been used to calibrate a number of microscopes. Thus, the value per unit would be unique for each. The procedure is as follows:

(1) Unscrew the eye lens of a 10x ocular (this may be the top or bottom, depending on the model) and place the micrometer disk (engraved side down) within the ocular. Use lens paper to handle the disk, and try to keep all surfaces free of lint or dust. Replace the ocular containing the micrometer disk in the microscope.

(2) Place the calibrated micrometer on the stage and, with the low power (10x objective), focus on the calibrated scale. It will be possible to distinguish the difference between the 0.1- and 0.01-mm divisions.

(3) Adjust the stage micrometer so the “0” line on the ocular micrometer is lined up exactly on top of the “0” line on the stage micrometer.

(4) After these two “0” lines are lined up (without moving the stage micrometer), look to the right of the “0” lines for another set of lines that is superimposed. Find a set as far to the right of the “0” lines as possible (the distance will vary with different objectives).

(5) Count the number of ocular divisions between the “0” lines and the point where the second set of lines is superimposed. Count the number of 0.1-mm divisions between the “0” lines and the second set of superimposed lines on the stage micrometer.

(6) Calculate the number of millimeters that is measured by a single, small ocular unit:

*Example:* 

\[
\frac{0.20 \text{ mm}}{27 \text{ ocular units}} = 0.0074 \text{ mm/ocular unit}
\]

To convert mm to \(\mu\)m, multiply by 1000:
The Quality System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in the most current edition of CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*. The quality management system approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any healthcare service’s path of workflow (i.e., operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager’s guide. The quality system essentials (QSEs) are:


M28-A2 addresses the quality system essentials (QSEs) indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI/NCCLS Publications section on the following page.

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI/NCCLS document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

M28-A2 addresses the clinical laboratory path of workflow steps indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI/NCCLS Publications section on the following page.

Adapted from CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*. ©Clinical and Laboratory Standards Institute. All rights reserved.
Related CLSI/NCCLS Publications

GP2-A4  Clinical Laboratory Technical Procedure Manuals; Approved Guideline—Fourth Edition (2002). This document provides guidance on development, review, approval, management, and use of policy, process, and procedure documents in the laboratory testing community.

GP5-A2  Clinical Laboratory Waste Management; Approved Guideline—Second Edition (2002). Based on U.S. regulations, this document provides guidance on safe handling and disposal of chemical, infectious, radioactive, and multihazardous wastes generated in the clinical laboratory.


M15-A  Laboratory Diagnosis of Blood-borne Parasitic Diseases; Approved Guideline (2000). This document contains guidelines for specimen collection, blood film preparation, and staining procedures. Recommendations for optimum timing of specimen collection to assist laboratories in detecting and identifying certain parasites are also included.

M29-A3  Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005). Based on U.S. regulations, this document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; 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.

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

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