

6TH EDITION

DIAGNOSTIC MEDICAL PARASITOLOGY

LYNNE SHORE GARCIA



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Washington, DC

Cover: Dog tapeworm (*Taenia pisiformis*), photograph taken using a light microscope, showing scolex with hooks. Spike Walker, Wellcome Images.

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Dedication

As with the first five editions, I dedicate this book to Marietta Voge, a truly rare individual who was widely recognized as one of the world's leading parasitologists. During her years as a diagnostic and research parasitologist at the University of California, Los Angeles, she touched the lives of many students and staff in a very special way. She was always more than willing to share her expertise with all who asked and volunteered this help over the years whenever contacted. She was always willing to donate a considerable amount of her personal time as a volunteer for various medical projects throughout the world.

She was a very special individual to work with, always interested in the person as well as the problem at hand. Her areas of teaching extended far beyond science. Whatever subject she was interested in received her total enthusiasm and dedication, and she had an exceptional ability to deal with detailed work. Her sense of fairness and professional integrity were remarkable; these ideals were shared with all who came in contact with her.

Her contributions to the field of diagnostic parasitology were numerous and included many classes, seminars, papers, and textbooks. The importance of working with Dr. Voge is hard to put into words. She was unique in her ability to allow a student to grow, both scientifically and personally. She could guide without constraints, teach without formal lectures, counsel without being judgmental, challenge without being unrealistic, tease without being cruel, and always be supportive regardless of the situation. She expected much from her students and employees and yet always gave considerably more than she received.

Scientific information gained from our association with her was invaluable; however, her impact on our lives was considerably more than scientific. She was always available for consultations and just to talk. She left all of us with a sense of having personally matured as a result of knowing and working with her over the years. She is missed by all of us, and yet her contributions in terms of teaching, consultations, volunteer work, professionalism, and friendship will remain with us forever.

I would also like to dedicate the sixth edition of this book to the bench technologists, those of you who provide critical diagnostic information on a daily basis and contribute such valuable input for excellent patient care.

Academic training provides key information in the field, but those who perform routine work at the bench often contribute much more than simple diagnostic identifications. Congratulations and thanks to all of you.

Finally, I also dedicate this book to John Lawrence. He was an extraordinary individual, and without his original encouragement and assistance, the first edition of the book would never have been written.

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Preface

During the past few years, the field of diagnostic medical parasitology has seen dramatic changes, including newly recognized parasites, emerging pathogens in new geographic areas, bioterrorism considerations and requirements, alternative techniques required by new regulatory requirements, reevaluation of diagnostic test options and ordering algorithms, continuing changes in the laboratory test menus, implementation of testing based on molecular techniques, reporting formats and report comments, coding and billing requirements, managed-care relevancy, increased need for consultation and educational initiatives for clients, and an overall increased awareness of parasitic infections from a worldwide perspective. We have seen organisms like the microsporidia change from the status of “unusual parasitic infection” to being widely recognized as among the most important infections in both immunocompetent and compromised patients. With confirmation of the fifth human malaria, *Plasmodium knowlesi*, this field has expanded dramatically. More sensitive diagnostic methods for organism detection in stool specimens are now commercially available for *Entamoeba histolytica*, *Entamoeba histolytica/E. dispar*, *Giardia lamblia*, *Cryptosporidium* spp., and *Trichomonas vaginalis*. Reagents are actively being developed for other organisms such as *Dientamoeba fragilis*, *Blastocystis* spp., and the microsporidia. We have seen *Cyclospora cayetanensis* coccidia become well recognized as the cause of diarrhea in immunocompetent and immunocompromised humans. We continue to see new disease presentations in compromised patients; a good example is granulomatous amebic encephalitis caused by *Acanthamoeba* spp., *Sappinia diploidea*, and *Balamuthia mandrillaris*. With the expansion of transplantation options, many parasites are potential threats to patients who are undergoing immunosuppression, and these must be considered within the context of this patient group. Transfusion transmission of potential parasitic pathogens continues to be problematic. Transfusion in general is becoming more widely recognized as a source of infection, and donors are also more likely to come from many parasite-endemic areas of the world. It is also important to recognize the many neglected parasitic infections seen within the United States; indeed, the world continues to shrink in terms of infectious diseases.

With expanding regulatory requirements related to the disposal of chemicals, laboratories are continuing to review the use of mercury compounds as specimen fixatives and learning to become familiar with organism morphology when using substitute compounds. Permanent staining of fecal smears confirms

that none of the substitute fixatives provide results of the same quality found with the use of mercuric chloride-based fixatives. However, the key issue is whether the intestinal parasites can be identified using these alternative fixatives, not how “perfect” they look. Many fixative options are now available, including single-vial collection systems, some of which are coupled with their own stains. Requirements also mandate that any laboratory using formalin must have formalin vapor monitored as both an 8-hour time-weighted average and 15-minute readings. Most laboratories are now familiar with the regulations on protection of health care workers from blood and other body fluids and have implemented specific changes that are no longer optional. Although laboratories were already using many of the safety recommendations, these regulations delineate in detail what must be done and documented. Regulatory information based on new shipping requirements is also included.

On the basis of excellent suggestions and comments, I have made the following changes in this new edition: (i) the chapter on case histories has been expanded and contains a large number of parasite medical case histories (case history, study questions, correct answer and discussion, and illustrative material); (ii) some of the life cycles have been redrawn, and new life cycles have been added; (iii) algorithms have been expanded; (iv) new tables and figures have been added throughout the book; (v) additional drawings and photographs have been added; (vi) extensive color images have replaced the black and white images; (vii) extensive updated text information is included, all of which was taken from a comprehensive literature review of all aspects of diagnostic medical parasitology; (viii) additional examples of unusual parasitic infections are included; (ix) the chapter on arthropods has been expanded and includes additional photographs and drawings and expanded text; (x) the chapter on the immunology of parasitic infections has been enlarged, and updated information on both antigen and antibody detection methods continues to be included in this edition; (xi) the chapter on histological identification of parasites has been dramatically expanded with diagrams of various parasites and their visual presentations in tissue sections, with greatly enhanced legends for all images; (xii) diagnostic methods using newer immunoassay and “dipstick” technology are included; and (xiii) the chapter on quality control has been expanded to include information on instrumentation and equipment, safety regulations, quality control and quality systems information, continuous quality improvement, and managed-care considerations. The appendixes have been expanded to contain more information on artifacts; expanded lists and photographs of products and commercial suppliers; algorithms for ordering specific tests that complement the ova and parasite examination; flowcharts for processing stool specimens; quality control recording sheets for use in the laboratory; and general references and relevant web sites. One of the most important expanded areas of the sixth edition is found in Appendix 7, which contains information that has been published within months prior to the final printing of this edition. This “late-breaking” synopsis of very recent publications can assist the reader in having access to the latest information available. I encourage you to review this section as you read various chapters throughout the book. A more comprehensive discussion of molecular methods has also been added to the sixth edition and can be found in Appendix 8. Appendix 9 contains comprehensive information on the most frequently asked questions for all aspects of human parasitology, and Appendix 10 contains information related to CPT coding for testing options for diagnostic parasitology.

The approach to the sixth edition of the book has been revised to present the diagnostic methods first, then the didactic discussion of parasitic infections

as the second component of the book. This change was made to ensure that the most recent and relevant material would be updated right before editing. My objective is to provide the user with clear, concise, well-organized, clinically relevant, cost-effective, and practical quality procedures for use in the clinical laboratory setting. To use and fully understand these methods for the parasites discussed, it is imperative that the user also understand information related to life cycle, morphology, clinical disease, pathogenesis, diagnosis, treatment, epidemiology, and prevention. My intent is to provide a comprehensive discussion of both aspects of the field of diagnostic medical parasitology: first, relevant diagnostic methods designed to detect and identify the organisms present, and second, a comprehensive discussion of the individual parasites. I believe that the book fulfills these objectives and provides readers, whether they are laboratorians, physicians, or other health care professionals, with not only comprehensive, but very practical information.

It is also important for readers to understand that there are many diagnostic test options available to the clinical laboratory; not every laboratory will approach the diagnosis of parasitic infections in the same way. The key to quality and clinically relevant diagnostic work is a thorough understanding of the pros and cons of each option and how various options may or may not be relevant for one's particular geographic area, laboratory size and range of expertise, client base, number and type of patients seen, personnel expertise and availability, equipment availability, educational initiatives, and communication options, just to name a few variables. However, it is also important to understand the regulations and technical recommendations that govern and guide this type of laboratory work; many of these guidelines are related to coding and reimbursement, proficiency testing, and overall clinical relevance.

The use of product names is not intended to endorse specific products or to exclude substitute products. Also, because of possible advances and changes in the therapy of parasitic infections, independent verification of drugs and drug dosages is always recommended. The diagnostic procedures are intended for laboratory use only by qualified and experienced individuals or by the personnel under their direct supervision. Every effort has been made to ensure accuracy; however, ASM Press and I encourage you to submit to us any suggestions, comments, and information on errors found.

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PART I

**Diagnostic
Procedures**

1

Philosophy and Approach to Diagnostic Parasitology

With the expansion of world travel and increased access to more varied geographic areas and populations, medical and laboratory professionals will continue to see more “tropical” diseases and infections in nonendemic areas. This is due to the rapidity with which both people and organisms can be conveyed from one place to another. Travel has become available and more affordable for many people throughout the world, including those who are in some way compromised in terms of their overall health status. The increased transportation of infectious agents, as well as potential human carriers, has been clearly demonstrated, particularly via air travel. It has also been well documented that vectors carrying parasitic organisms can be transported via air travel in baggage and in the unpressurized parts of the plane itself; once released, these infected vectors can then transmit these parasites to humans, even in nonendemic areas.

With the continued increase in the number of patients whose immune systems are compromised through either underlying illness, chemotherapy, transplantation, AIDS, or age, we are much more likely to see increasing numbers of opportunistic infections, including those caused by parasites. Also, we continue to discover and document organisms once thought to be nonpathogenic that, when found in the compromised host, can cause serious disease. In considering the potential causes of illness in this patient population, the possibility of parasitic infections must always be considered as part of the differential diagnosis.

Diagnostic procedures in the field of medical parasitology require a great deal of judgmental and interpretative experience and are, with very few exceptions, classified by the Clinical Laboratory Improvement Act of 1988 (CLIA '88) as high complexity procedures. Very few procedures are automated, and organism identification relies primarily on morphologic characteristics that can be very difficult to differentiate. Although parasite morphology can be “learned” at the microscope, knowledge about the life cycle, epidemiology, infectivity, geographic range, clinical symptoms, range of illness, disease presentation depending on immune status, and recommended therapy is critical to the operation of any laboratory providing diagnostic services in medical parasitology.

The basic approach to diagnostic parasitology should be no different from that used in other areas of microbiology. There are guidelines published by the American Society for Microbiology (1–5), the American Society of Parasitologists (6), the American Society for Medical Technology (7), the College of American Pathologists (8), and the Clinical and Laboratory Standards Institute (formerly

National Committee for Clinical Laboratory Standards) (9–16) that contain recommended procedures for this field. If these general guidelines and recommendations are not followed, there is some question as to the qualifications of the laboratory performing the diagnostic work. At the very least, the clinician should be informed about the limitations of the procedures that are being used. These guidelines are also accompanied by specific regulations for a number of laboratory issues and include CLIA '88 and requirements related to safety and protection of employees from blood or blood-borne pathogens (standard precautions) (17–20).

Because it is difficult for medical staff to maintain expertise in every available diagnostic procedure within microbiology, it is mandatory that close communication exist between the laboratory and clinicians. Frequent and complete communication, particularly concerning appropriate test orders and the clinical relevance of any diagnostic procedure within the context of total patient care and quality assurance, is very important. Therapeutic intervention often depends on results obtained from these procedures; therefore, the clinician must be aware of the limitations of each test method and the results obtained. This information becomes particularly important when one is discussing the patient's history and the recommended number and types of specimens to be submitted for examination.

During the past few years, there has been an increased awareness of the importance of having trained and qualified personnel perform these diagnostic procedures. There has been a concerted effort among many individuals and institutions in this country to upgrade the level of teaching and to bring to the medical community's attention the need for individuals who are familiar with diagnostic parasitology. With many laboratories decreasing staff size as a cost-containment measure, we are also seeing more "generalists" who are rotating throughout many sections of the laboratory, not just microbiology. Although necessary because of managed-care constraints and continued growth of capitated contracts, this approach contributes to the difficulties in maintaining well-trained staff in some of the specialty areas of microbiology. It becomes even more important to provide well-written laboratory protocols and to standardize test methods for consistency. There has also been increased awareness within the medical community of the need for additional training in the area of infectious diseases for the clinician and laboratory technician alike. This need has been reflected in the number of workshops, seminars, and publications that are available. The integration of information among all members of the health care team has certainly improved in terms of overall patient care.

The field of microbiology has taken on additional relevance and importance for a number of other reasons. Improved means of travel has made the world a smaller place. An individual's chances of exposure to parasites not endemic to his or her homeland and the possibility of acquiring or transmitting certain infections have been increasing. These facts emphasize the need to take a correct and complete history from a patient. It is important to be aware of the organisms commonly found within certain areas of the world and the makeup of the patient population being serviced at any particular health facility.

The most important step in the diagnosis of parasitic infections is the selection and submission of the appropriate clinical specimen within specified time lines and according to set protocols (21–23).

It is also important for the physician to know the efficacy of any diagnostic technique for parasite recovery and eventual diagnosis. Our approach to testing is undergoing continuous review, particularly within the current health care environment and cost-containment initiatives. The issue of patient care becomes particularly important when we begin to examine the number and types of compromised patients now being seen in all facilities. The increased publicity concerning immunocompromised patients has led to a greater awareness of parasitic infections in this patient population, regardless of the original cause of the immune deficiencies. Many of these patients with immune system defects are particularly at risk, whether because of previously acquired infections that have remained latent for many years or because of susceptibility to new infections. Many of these infections may present with unusual symptoms, and some are relatively new disease entities or those that are less commonly encountered (microsporidiosis, granulomatous amebic encephalitis, and infection with *Cyclospora cayetanensis*).

Often in other areas of microbiology, therapy is begun on the basis of patient history and symptoms. This approach is generally not recommended or used in cases of parasitic infection. Thus, the understanding of the characteristics of any parasitic infection (general geographic range, life cycle, clinical disease, diagnostic methods, therapy, epidemiology, and control) and the use of appropriate diagnostic procedures accompanied by a complete understanding of the limitations of each procedure become very important. Because of this approach to patient care, the general consensus among individuals within the field of diagnostic medical parasitology is that the use of certain incomplete procedures may result in incorrect information for the physician and may ultimately compromise patient care.

The main emphasis should be on the importance of understanding and recognizing potential parasitic infections, submitting the appropriate number and type of clinical

specimens, knowing what procedures may provide confirmation of the diagnosis, and recognizing the implications and limitations of information provided to the physician. With the current emphasis on the development and use of molecular methods, understanding the benefits and limitations of these procedures will be critical to patient care outcomes. If there is an incomplete understanding of the requirements for high-quality diagnostic testing, incomplete information will be transmitted to the clinician. It is the responsibility of both the laboratory and the clinician to develop a greater awareness of the importance of these requirements.

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2

Collection, Preservation, and Shipment of Fecal Specimens

Safety

Fresh-specimen collection

Collection of the specimen

Number of specimens to be collected (standard recommendation)

Number of specimens to be collected (pros and cons of various options)

Collection times

Specimen type, specimen stability, and need for preservation

Preservation of specimens

Preservatives

Formalin

MIF

SAF

Schaudinn's fluid

Schaudinn's fluid containing PVA (mercury base)

Schaudinn's fluid containing PVA (copper base, zinc base)

Single-vial collection systems (other than SAF)

Universal Fixative (TOTAL-FIX)

Use of fixatives

Quality control for stool fixatives

Procedure notes for use of preservatives

Procedure limitations for use of preservatives

Shipment of diagnostic specimens, biological products, etiologic agents, or infectious substances

Documentation

This chapter discusses various collection methods that are available for specimens suspected of containing parasites or parasitic elements. When a laboratory selects its collection methods, the decision should be based on a thorough understanding of the value and limitations of each. One of the most important aspects of specimen collection is that the final laboratory results based on parasite recovery and identification will depend on the initial fixation of the organisms (1–3). Unless the appropriate specimens are properly collected and processed, these infections may not be detected (1). Considering the current era of cost containment and review of clinical relevance of laboratory information generated, specimen rejection criteria have become more important within the context of all diagnostic microbiology procedures. Diagnostic laboratory results based on improperly collected specimens may require excessive expenditures of time and supplies and may also mislead the physician. As a part of any overall total quality management or continuous quality improvement program for the laboratory, the generation of test results must begin with stringent criteria for specimen acceptance or rejection.

Clinically relevant diagnostic parasitology testing also depends on receiving appropriate test orders from the physician. Depending on the patient's clinical condition and travel history, very specific diagnostic tests may be recommended. It is extremely important that physician clients are aware of the test order options available within the laboratory test menu and the pros and cons of each test when considered within the context of the patient's history and symptoms. Without the proper test orders, diagnostic test results may be misleading or actually incorrect. Appropriate and complete communication regarding test orders between the laboratory and physicians is mandatory for high-quality patient care.

Safety

All fresh specimens should be handled carefully, since each specimen represents a potential source of infectious material (bacteria, viruses, fungi, and parasites). Safety precautions should include awareness of the following: proper labeling of fixatives; specific areas designated for specimen handling (biological safety cabinets may be necessary under certain circumstances); proper containers for centrifugation; acceptable discard policies; appropriate policies for no eating,

drinking, or smoking, etc., within the working areas; and, if applicable, correct techniques for organism culture and/or animal inoculation.

Since diagnostic parasitology work is most often performed within the microbiology division of a clinical laboratory, all general guidelines for safety would also apply. Any special precautions which apply to a particular technique are discussed in the following chapters. In general, standard precautions as outlined by the Occupational Safety and Health Act must be followed when applicable, particularly when one is handling blood and other body fluids (4).

Fresh-Specimen Collection

Procedures for the recovery of intestinal parasites should always be performed before barium is used for radiological examination. Stool specimens containing barium are unacceptable for examination, and intestinal protozoa may be undetectable for 5 to 10 days after barium is given to the patient. There are also certain substances and medications that interfere with the detection of intestinal protozoa: mineral oil, bismuth, antibiotics, antimalarial agents, and nonabsorbable antidiarrheal preparations. After administration of any of these compounds, parasitic organisms may not be recovered for a week to several weeks. The two most commonly used substances are barium and antibiotics, such as tetracycline, which modify the gastrointestinal tract flora. Specimen collection should be delayed for 5 to 10 days or at least 2 weeks after barium or antibiotics, respectively, are administered (1, 2, 5–24). The use of antibacterial therapy that affects the normal gastrointestinal tract flora will diminish the numbers of protozoa, since they feed on intestinal bacteria.

Collection of the Specimen

Fecal specimens should be collected in clean, wide-mouth containers; often a 0.5-pint (ca. 0.24-liter) waxed cardboard or plastic container with a tight-fitting lid is selected for this purpose. The fit of the lid is particularly important, both from the standpoint of accidental spillage and in order to maintain moisture within the specimen. The specimens should not be contaminated with water or urine, because water may contain free-living organisms that can be mistaken for human parasites and urine may destroy motile organisms. For safety reasons, stool specimen containers should be placed in plastic bags when transported to the laboratory for testing. Fresh specimens can also be submitted in collection vials (Fig. 2.1). All fresh specimens should be carefully handled, since they are potential sources of infectious organisms, including bacteria, viruses, and parasites. Every specimen should be identified with the following



Figure 2.1 Stool collection vial; “clean vial” contains no fixatives. doi:10.1128/9781555819002.ch2.f2.1

minimal information: patient’s name and identification number, physician’s name, and the date and time the specimen was collected (if the laboratory is computerized, the date and time may reflect arrival in the laboratory, not the actual collection time). The specimen must also be accompanied by a request form indicating which laboratory procedures are to be performed. It is also very helpful to have information concerning the presumptive diagnosis or relevant travel history; however, this information is rarely available, and under certain circumstances, the physician will have to be contacted for additional patient history (Example: Fever of unknown origin [FUO]—possible malaria).

Number of Specimens To Be Collected (Standard Recommendation)

It is recommended that a normal examination for stool parasites before therapy include three specimens, consisting of two specimens collected from normal movements and one collected after the use of a cathartic such as magnesium sulfate or Fleet’s Phospho-Soda. A cathartic with an oil base should not be used, and a stool softener (taken either orally or as a suppository) is usually inadequate for obtaining a purged specimen. The purpose of the laxative is to stimulate some “flushing” action within the gastrointestinal tract, possibly allowing one to obtain more organisms

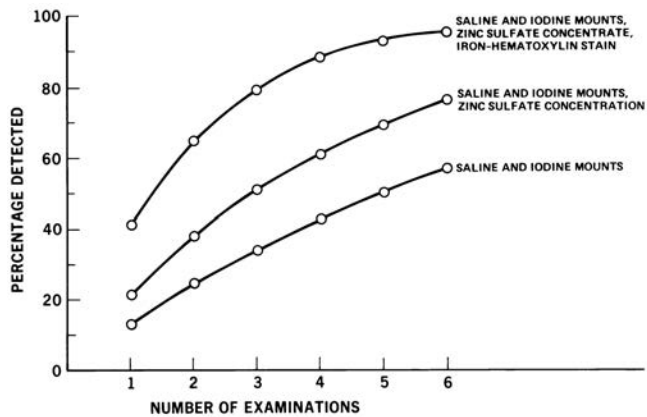


Figure 2.2 Increased detection of *Entamoeba histolytica* by using various diagnostic techniques and serial stool specimens. (Adapted by Markell EK, Voge M, John DT, 1992, *Medical Parasitology*, 7th ed., The W. B. Saunders Co., Philadelphia, PA, from references 17 and 31, with permission.)
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for recovery and identification. Obviously, if the patient already has diarrhea or dysentery, the use of any laxatives would be contraindicated. Since the majority of patients are symptomatic prior to submission of stool specimens for examination, the need for a laxative is relatively uncommon.

When a patient is suspected of having intestinal amebiasis, six specimens may be recommended. The examination of six specimens ensures detection of approximately 90% of amebic infections (5) (Fig. 2.2). However, because of cost-containment measures, the examination of six specimens is rarely requested.

Three specimens are also recommended for posttherapy examinations, and they should be collected as outlined above. However, a patient who has received treatment for a protozoan infection should be checked 3 to 4 weeks after therapy, and those treated for *Taenia* infections should be checked 5 to 6 weeks after therapy. In some cases, the physician will assume a cure for tapeworm infection unless proglottids reappear in the stool; therefore, no posttherapy specimens are submitted for examination.

Number of Specimens To Be Collected (Pros and Cons of Various Options)

During the past few years, a number of issues have surfaced regarding the collection, processing, and testing of stool specimens for diagnostic parasitology. Many of the new suggestions and options have arisen as a result of continued cost-containment measures, limited reimbursement, and the elimination of mercury-based compounds for stool preservatives. The number of nonmercury preservative choices, collection systems, concentration devices, and immunoas-

says has increased dramatically. Many laboratories continue to review the options, and some may be having difficulties in selecting the proper approach (7–22).

It is important to realize that there are many acceptable options and that many laboratories will select different approaches. These differences should not be categorized as “right or wrong” or “acceptable or unacceptable”—they are merely different! To assume that there is only one correct approach for the examination of stool specimens is neither appropriate nor realistic. There are many parameters to consider before selecting the approach for your own laboratory. In no particular order, some of the considerations include client base, physician ordering patterns, number of specimens received per month, cost, presence or absence of appropriate equipment, current and possible methodologies (including immunoassays such as enzyme immunoassay [EIA], fluorescent-antibody assay [FA], and rapid membrane flow cartridge devices [rapids]), availability of expert microscopists, collection options, selection of preservative-stain combinations, reimbursement issues, client education, area of the world where laboratory is located, and emphasis on the most common infections (helminth or protozoa or both).

When considering available options and laboratory test menus, it is important to make sure that the pros and cons of the approaches selected are thoroughly understood and that diagnostic tests, potential results, and reporting formats are carefully explained to all clients. As an example, if the results of a stool examination are based on a concentration sediment examination only, this information must be conveyed to the physician. Many of the intestinal protozoa are missed by this diagnostic test approach, and it is important for the physician to recognize the limitations of such testing. Most physicians receive very little, if any, exposure to medical parasitology in medical school, and many newer physicians trained as generalists or family practitioners also have limited parasitology training or experience.

In most cases, it is probably realistic to assume that patients are symptomatic if they are submitting stool specimens for diagnostic parasitology testing. In an excellent article by Hiatt et al., the premise tested was that a single stool sample from a symptomatic patient would be sufficient to diagnose infections with intestinal protozoa (23). However, with additional stool examinations for symptomatic patients, the yield of intestinal protozoa increased dramatically (*Entamoeba histolytica*, 22.7% increase; *Giardia lamblia*, 11.3% increase; and *Dientamoeba fragilis*, 31.1% increase). This publication again demonstrates the problems with performing only a single stool examination (using the ova and parasite examination). If the patient becomes asymptomatic after the first stool examination,