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Best Practices in Parasitology

Addressing Common Diagnostic Challenges

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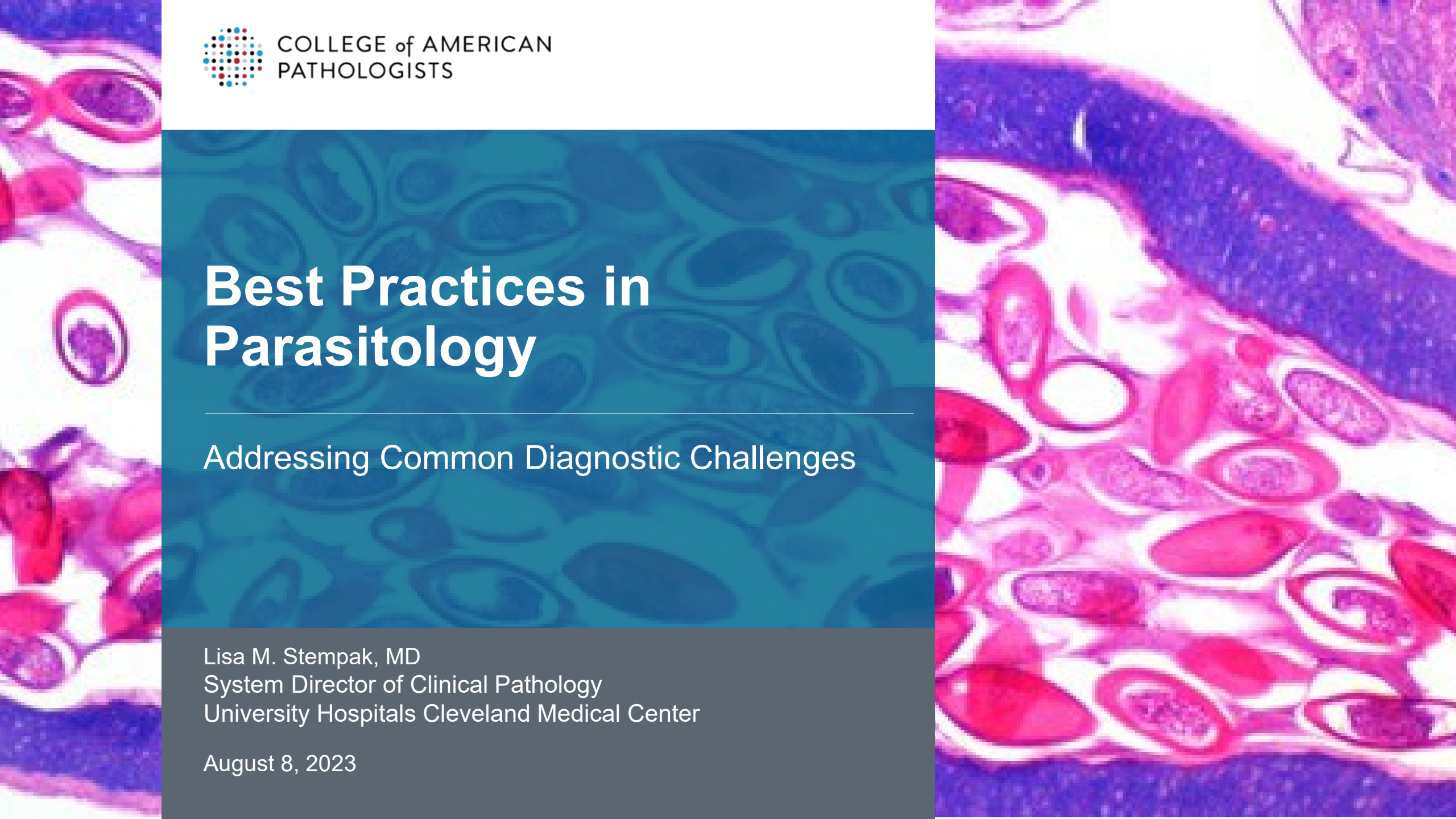

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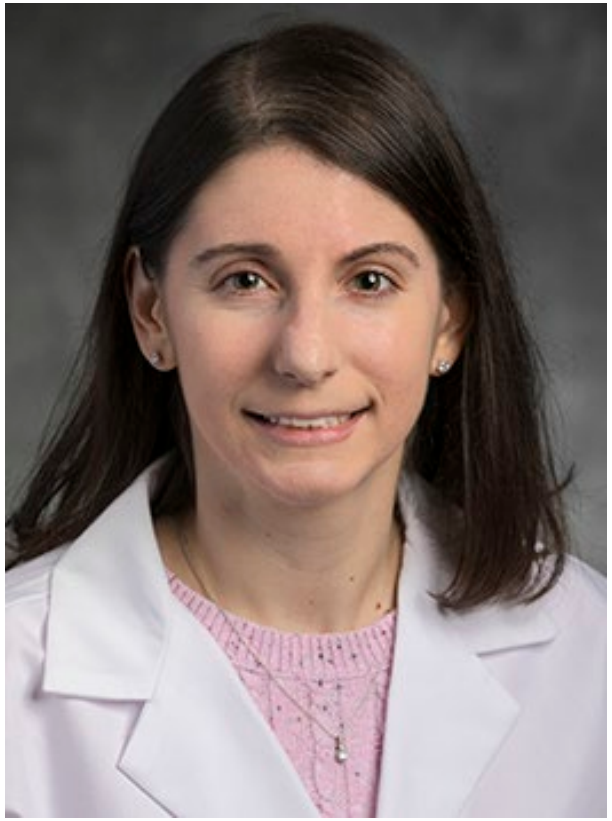

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Lisa Stempak, MD, FCAP

- Advisor for Cytovale Inc.

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Objectives

- Describe best practices for the detection of parasites in the stool and blood
- Understand how the College of American Pathologists (CAP) checklist requirements support the accurate diagnosis of parasites
- Identify high-quality reference materials to aid in parasite identification

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Introduction

- The diagnosis of parasites can be challenging
  - Often these organisms are encountered infrequently
  - Difficult matrix, multiple staining techniques, examination of numerous microscopic fields
  - Require diagnostic morphologic expertise
- Accurate identification is important
  - Obligation to ensure high-quality patient care
  - Allows for selection of appropriate treatment
  - Appropriate follow up testing if required

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Best Practice 1: Proper Specimen Collection (Pre-Analytic)

Important steps in the diagnosis of intestinal parasites

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Best Practice 1: Checklist Requirement

MIC.52190 **Stool Number/Timing** **Phase I**

The laboratory defines the appropriate number and/or timing of collection of stool specimens submitted for routine parasitology testing.

NOTE: The laboratory may develop policies with its clinicians for the number and/or timing of collection of stool specimens submitted for routine parasitology testing.

Suggestions made by the authors of a 1996 CAP Q-Probes study (Valenstein et al) include:

1. *Accept no more than two or three specimens/patients without prior consultation with an individual who can explain the limited yield provided by additional specimens*
2. *Do not accept specimens from inpatients after the fourth hospital day, without prior consultation*

These recommendations are for diagnostic testing. Different policies may apply to tests ordered for follow-up.

Source 1

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Best Practice 2: Microscopic Exam (Analytic)

- Components of an ova and parasite (O&P) exam:
  - Direct wet preparation- optional
  - Concentration procedures- required
  - Permanent stained smear- required
- The two parts of the O&P exam are complimentary and when used together provide a comprehensive approach to obtain the correct diagnosis
- Referral to complete testing by reference laboratory is required if both portions aren't performed in-house

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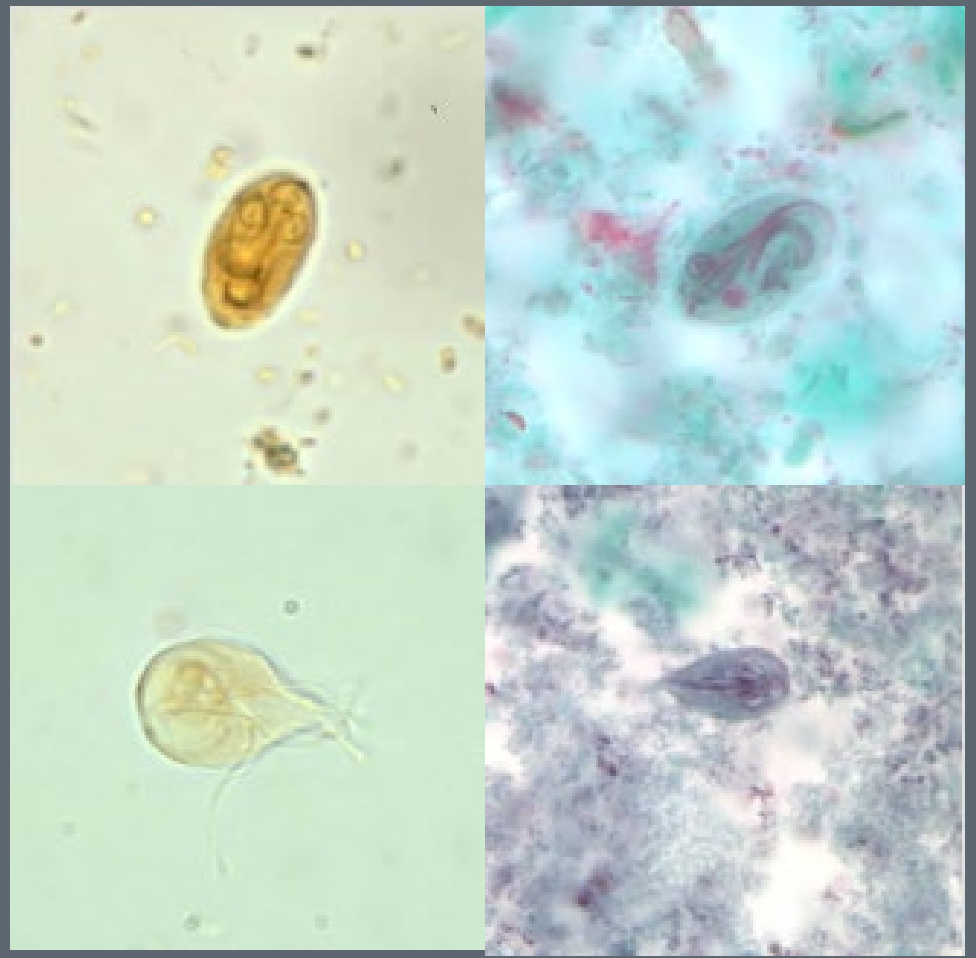
Best Practice 2: Example

Giardia duodenalis

- Cyst
  - Ovoid shape, 8-20 µm
  - 4 nuclei, intracytoplasmic fibrils
- Trophozoite
  - Pear shaped, 12-15 µm
  - 2 nuclei, rod-like structures ‘median bodies’
  - 8 flagella
- ‘Falling leaf motility’ on wet prep



Source 2

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Best Practice 2: Checklist Requirement

MIC.52100 **Ova/Parasite Exam** **Phase II**

The microscopic examination of all stools submitted for an ova and parasite (O&P) examination includes a concentration procedure and a permanent stain.

NOTE: When a stool specimen is submitted fresh, the usual approach would be to perform a direct wet preparation (looking for motility), a concentration (helminth eggs/larvae/protozoan cysts), and the permanent stained smear (identification of protozoa missed by concentration and confirmation of suspect organisms). As a minimum (and certainly if the stool is submitted in preservatives), the standard O&P examination would include the concentration procedure and a permanent stained smear. The main point is to ensure that the permanent stained smear is performed on all stool specimens, regardless of what was or was not seen in the concentration wet preparation. Often, intestinal protozoa will be seen in the permanent stained smear, but may be missed in the concentration examination. If the laboratory does not perform both a concentration procedure and a permanent stain, it must refer the testing that is not completed to a referral laboratory so that testing may be completed.

Evidence of Compliance:

- ✓ Patient reports/worksheets with concentration and permanent stain results

Source 1

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Best Practice 3: Accurate CAP Test Menu

- Include both the concentration procedure and the permanent smear on your laboratories test menu
  - ‘Parasite ID, fecal, direct and/or concentration’
  - ‘Parasite ID, fecal, permanent stain’
- An inaccurate or incomplete test menu is regularly the most frequently cited deficiency each year

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Best Practice 3: Checklist Requirement

COM.01200 Activity Menu **Phase I**

The laboratory's current CAP Activity Menu accurately reflects the testing performed.

NOTE: The laboratory's CAP Activity Menu must include all patient/client testing performed by the laboratory.

- *For laboratories with a CLIA certificate, it includes all testing and activities performed under that CLIA certificate.*
- *For laboratories not subject to CLIA, it includes all testing and activities meeting all of the following criteria: 1) performed under the same laboratory director, 2) under the same laboratory name, and 3) at the same physical premises (contiguous campus).*

The testing and activities must be listed on the laboratory's CAP Activity Menu regardless of whether it is also accredited by another organization. ***The laboratory must update its CAP Activity Menu when tests are added or removed by logging into e-LAB Solutions Suite on cap.org and going to Organization Profile - Sections/Departments.*** *In order to ensure proper customization of the checklists, the laboratory must also ensure its activity menu is accurate for non-test activities, such as methods and types of services offered.*

If an inspector identifies that a laboratory is performing tests or procedures not included on the laboratory's CAP Activity Menu, the inspector must do the following:

- *Cite COM.01200 as a deficiency*
- *Contact the CAP (800-323-4040) for inspection instructions as requirements may be missing from a laboratory's customized checklist*
- *Record whether those tests/procedures were inspected on the appropriate section page in the Inspector's Summation Report (ISR).*

Source 1

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Best Practice 4: Ocular Micrometer (Analytic)

- The identification of protozoa and helminth eggs often depends on an accurate measurement of size
- A calibrated ocular micrometer is required for clinical diagnosis of parasites
- Depending on the objective magnification used, the divisions in the micrometer represent different measurements
- Recalibration should occur at least yearly and after any microscope maintenance

Pro Tip: Measurement of red blood cells (~7.5 µm) is an easy way to confirm calibration is correct at common magnifications (100x, 400x, 1000x oil immersion)

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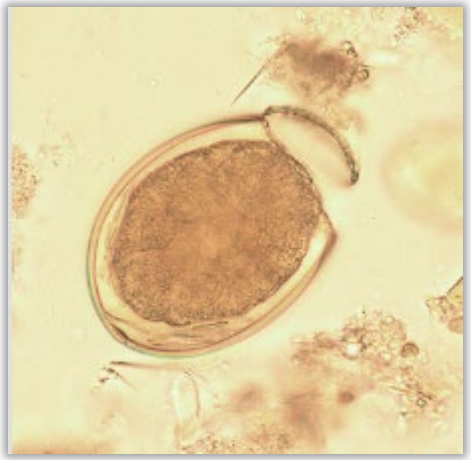
Best Practice 4: Example

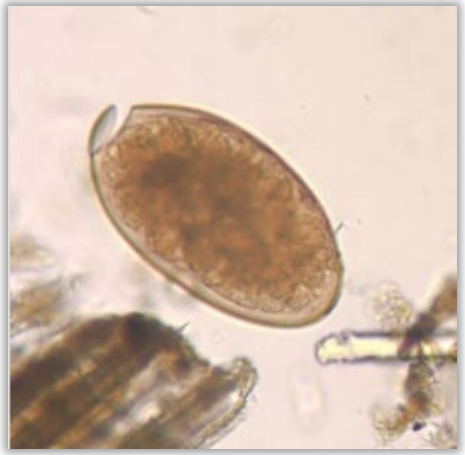
P3-21 Diph./Diboth./Aden. sp.



***Diphyllobothrium latum*:**

- Cestode (fish tapeworm)
- Oval eggs with operculum, small knob at other end
- Size- 55-75 µm by 40-50 µm

Fasciola/Fasc. buski eggs Unacceptable



***Fasciola* spp.**

- Trematode (liver fluke)
- Broadly ellipsoidal egg with operculum
- Size- 130-150 µm by 60-90 µm

Source 3

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Best Practice 4: Checklist Requirement

COM.30690 Calibration/Recalibration - Ocular Micrometer **Phase II**

The ocular micrometer (when required) is calibrated for the microscope(s) and the specific objective(s) with which it is used.

NOTE: An ocular micrometer is required for certain types of testing, including:

- *Parasitology identification when determining the size of eggs, larvae, cysts, trophozoites, and microfilaria or other bloodborne parasites*
- *Sperm morphology for use of certain sperm morphology classification methods (Kruger Strict and World Health Organization (WHO) methods references in the 3rd and 4th editions).*
- *Surgical pathology when accurate microscopic measurements are needed (eg, measuring depth or extent of invasion and margins in various cancers, extent of involvement in needle core biopsies, size of organisms or other microscopic findings)*

Calibrations must be checked against a calibrated stage micrometer slide or other object(s) of known dimensions appropriate to the use of the ocular micrometer.

Any change in the optics of the microscope (eg, change in objective or ocular lens) requires recalibration. If there are no changes to a particular microscope's optical components, there is no need to recheck calibration.

Evidence of Compliance:

✓ Records of initial calibration and recalibration, if applicable

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Best Practice 5: Reference Materials
(Interpretative)

- Technical procedures
  - Clinical Microbiology Procedures Handbook, 5th edition
  - M28-A2: 2005 Procedures for the Recovery and Identification of Parasites from the Intestinal Tract, 2nd Edition
  - M15-A: 2000 Laboratory Diagnosis of Blood-borne Parasitic Diseases, 1st Edition
- Altas/reference guides
  - Parasitology Benchtop Reference Guide: An Illustrated Guide for Commonly Encountered Parasites
  - Ash & Orihel's Atlas of Human Parasitology, 5th edition
  - Diagnostic Medical Parasitology, 6th edition

Source 4 and Source 5

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Best Practice 5: Reference Materials

(Interpretative), continued

- Website
  - Centers for Disease Control and Prevention- DPDx Laboratory Identification of Parasites of Public Concern: https://www.cdc.gov/dpdx/index.html
- CAP Participant Summary Reports
- Permanent smear reference sets
- Upcoming- CAP Parasitology Toolbox

Source 6

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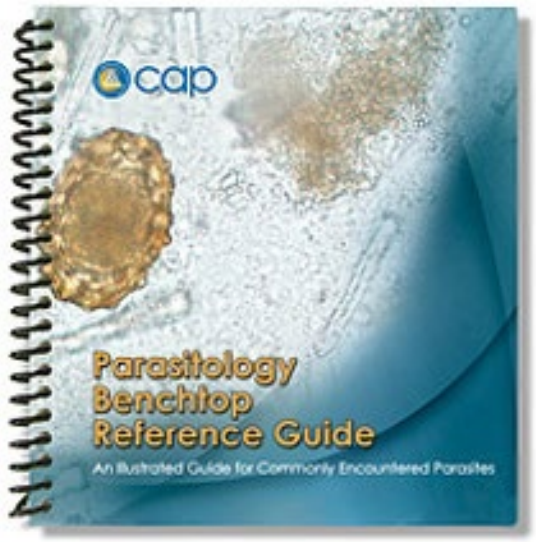
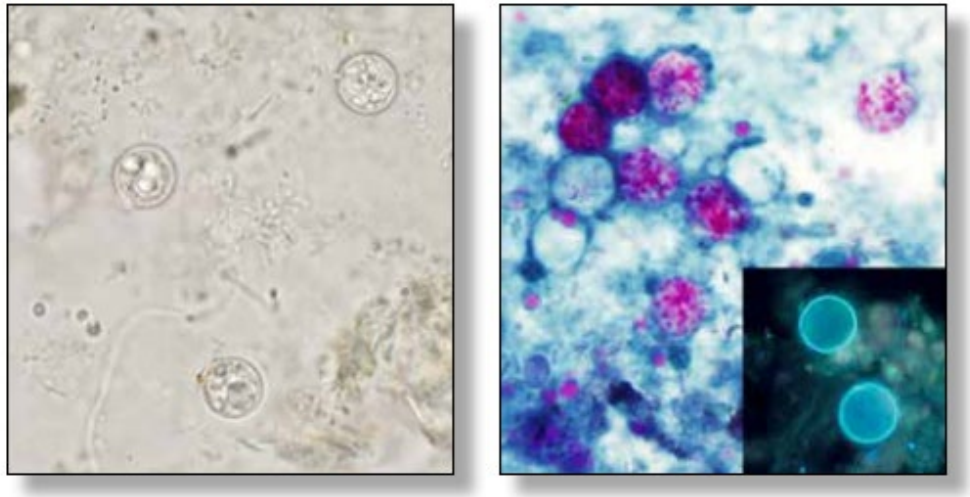
Best Practice 5: Reference Materials



Helminths

Cyclospora cayetanensis



Microscopic Morphology: Oocysts are round to oval and measure 7–10 µm in diameter (usual range 8–10 µm). They are shed in an unsporulated form so that internal sporozoites are not typically seen in stool unless there is a substantial delay in processing. Globular material is commonly seen in unsporulated forms in unstained wet preparations (above left). The oocysts are clear (unstained "ghost" cells) to pink or bright pink/red on modified acid-fast (above right) and modified safranin stains. Oocysts strongly autofluoresce under UV epifluorescence (above right, inset).

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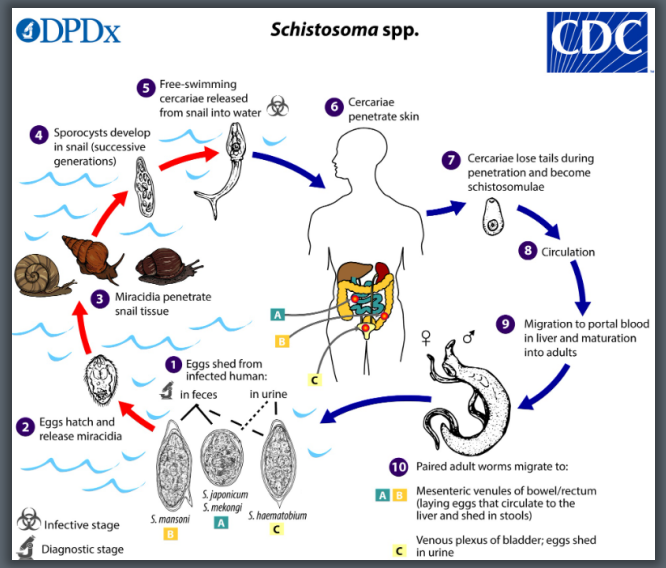
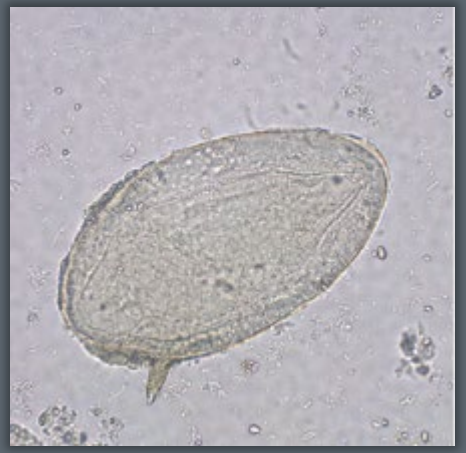
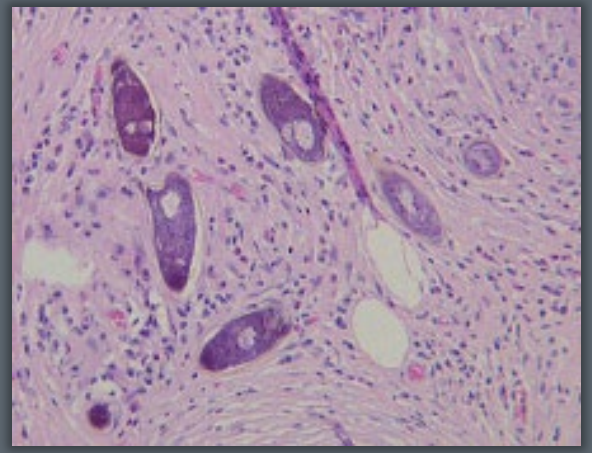
Best Practice 5: Reference Materials

DPDx Laboratory Identification of Parasites of Public Concern

- Educational resource designed for health professionals and laboratory scientists
- Contains information regarding parasite biology (life cycle), image gallery, laboratory diagnosis, and resources







Source 7 and Source 8

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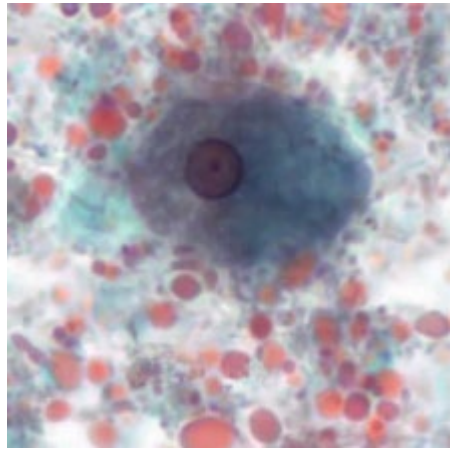
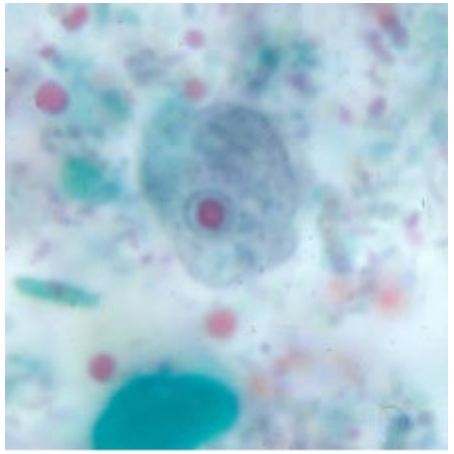
Best Practice: Example 1

Parasite ID	P-10	E. HIST/DISP/MOSH/BANG	ENDOLIMAX NANA E. NANA / I. BUETSCHLII	Unacceptable



Entamoeba histolytica/dispar

- Pathogenic amoeba
- Trophozoite with single central karyosome, uniformly distributed peripheral chromatin
- Size- 15-20 µm



Endolimax nana

- Non-pathogenic amoeba
- Trophozoite with a single characteristically large karyosome, nuclei lacks peripheral chromatin
- Size- 8-10 µm

Source 9 and Source 10

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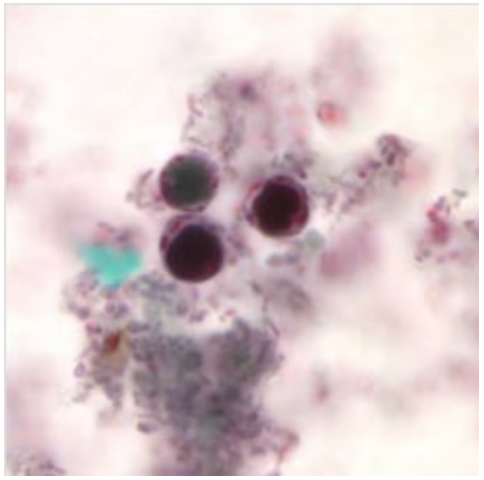
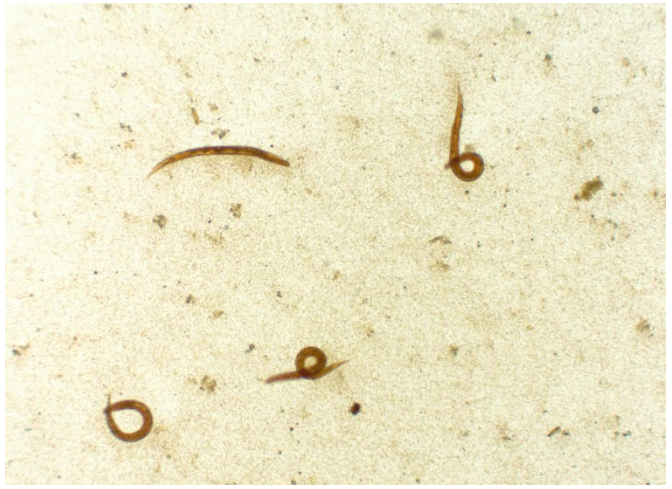
Best Practice 5: Example 2

Parasite ID	P-04	Blastocystis sp.	Strongyloides sterc. larv	Unacceptable





Blastocystis sp.

- Unicellular parasite, unclear pathogenic potential
- Spherical to oval vacuolar forms
- Size- 5-40 µm, humans 8-10 µm

Strongyloides stercoralis

- Nematode (roundworm)
- Rhabditiform larvae found in stool
- Eggs hatch in the mucosa of the small intestine
- Size- 180-380 µm

Source 11

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Best Practice 5: Checklist Requirement

MIC.51000	**Reference Materials**	**Phase I**
	Reference materials, such as permanent mounts, photomicrographs, CLSI documents M15-A and M28-A2, or printed atlases are available at the work bench to assist with identifications.	

Source 1

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Pro Tip: Parasite Identification

- Multiple sets of eyes are better than one
- Recommend a second technologist, supervisor, and/or medical director review positive slides to confirm accuracy of identification
- If staffing allows, two technologist to review negative slides as well

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Best Practice 6: Blood Parasite Detection

Blood parasites are considered to be potentially life threatening and blood smear examination and organism identification should be treated as STAT requests

Pro Tip: Patient geographical location/travel history is important

- Helpful in determining areas with malaria, common species, drug resistance, and recommended chemprophylaxis
- Centers for Disease Control and Prevention Malaria Information and Prophylaxis by Country, https://www.cdc.gov/malaria/travelers/country_table/i.html

Country	Areas with Malaria	Drug Resistance[2]	Malaria Species[3]	Recommended Chemoprophylaxis[4]	Key Information Needed and Helpful Links to Assess Need for Prophylaxis for Select Countries
Iceland	None	Not Applicable	Not Applicable	Not Applicable	
India	All areas throughout country, including cities of Bombay (Mumbai) and New Delhi, except none in areas > 2,000 m (6,562 ft) in Himachal Pradesh, Jammu and Kashmir, and Sikkim.	Chloroquine	*P. vivax* 50%, *P. falciparum* >40%, *P. malariae* and *P. ovale* rare	Atovaquone-proguanil, doxycycline, mefloquine, or tafenoquine[5]	1) City(ies) of travel 2) Altitude of city(ies) of travel 3) Province(s) of travel Altitude information and to determine if city is within a certain province Map of provinces in India

Source 12

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Best Practice 6: Checklist Requirement

MIC.52193	Blood Parasite Detection	Phase II
	The microscopic examination of blood films submitted for detection of blood parasites allows for detection of parasites responsible for malaria, babesiosis, trypanosomiasis and filariasis.	

- Thick and thin smears required for optimal sensitivity and speciation
- Timing collection appropriately to detect periodicity in blood (nocturnal)
- Important to first screen slides at low power to detect a blood microfilaria
- Calibrated ocular micrometer required for microfilaria and other bloodborne parasites

Source 1

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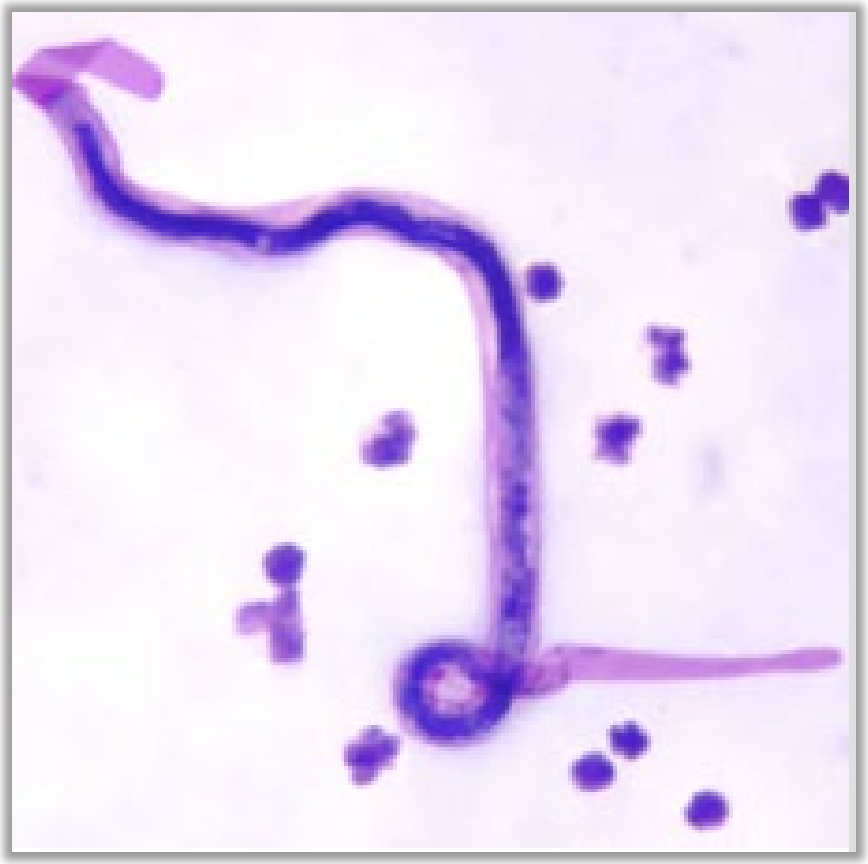
Best Practice 6: Example

Brugia malayi

- Microfilaria circulate in blood
- Sheathed, pink stained with Giemsa stain
- Tail is tapered with two discontinuous nuclei
- Nocturnal
- Size- 175 to 230 µm



Source 13

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Best Practice 7: Malaria Detection

- Giemsa stained blood films are the most reliable and efficient way to definitively diagnose nearly all blood parasites
- Both a thick and thin blood smear should be prepared and reviewed under oil immersion
  - Thick blood smear
    - Allows for the examination of a larger volume of blood, enhancing detection for 'immunologically naïve' patients who may be symptomatic at very low parasitemia levels
  - Thin blood smear
    - Clear red cell morphology that allows for confirmation and species level identification of *Plasmodium* spp. and *Babesia*
    - Percent parasitemia is calculated from thin smear

Pro Tip: The two most important questions for morphologic identification are

1. Is it malaria?
2. Is it *Plasmodium falciparum*?

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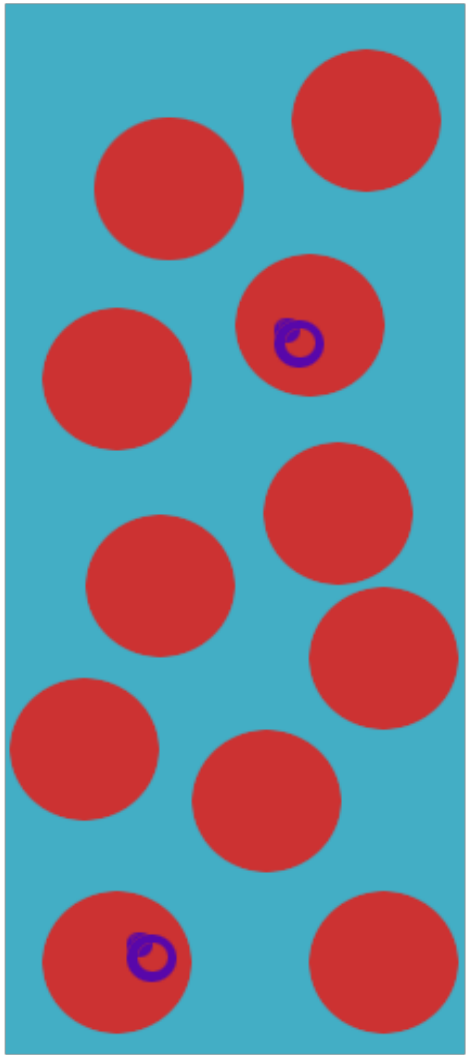
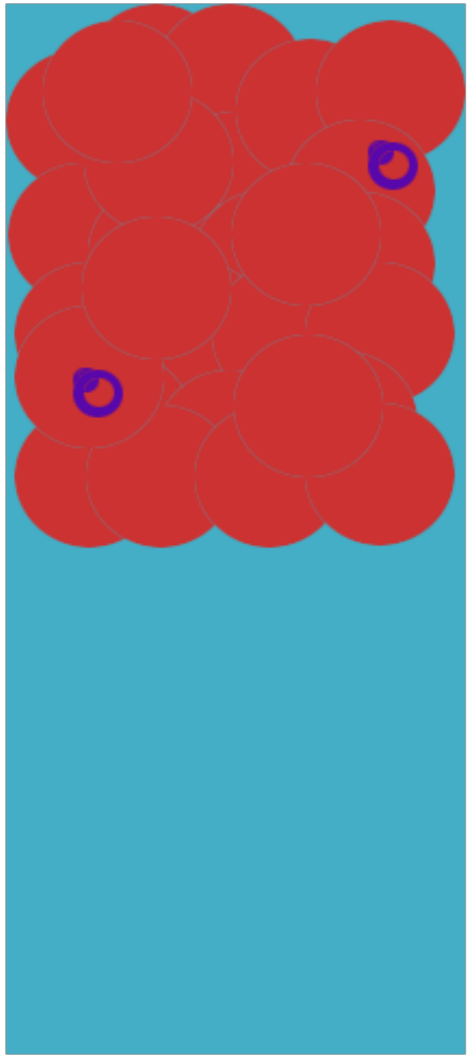
Thin & Thick Blood Smears

Thin





Thick

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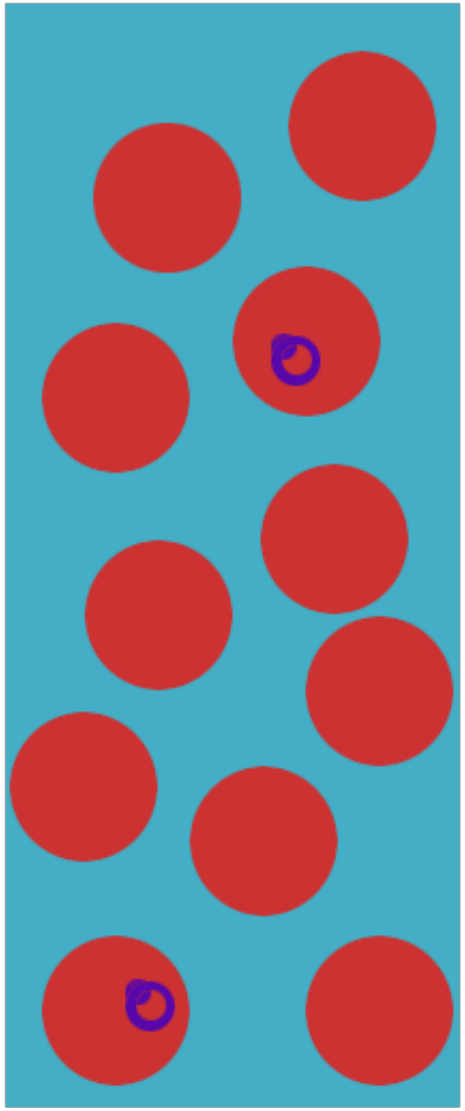
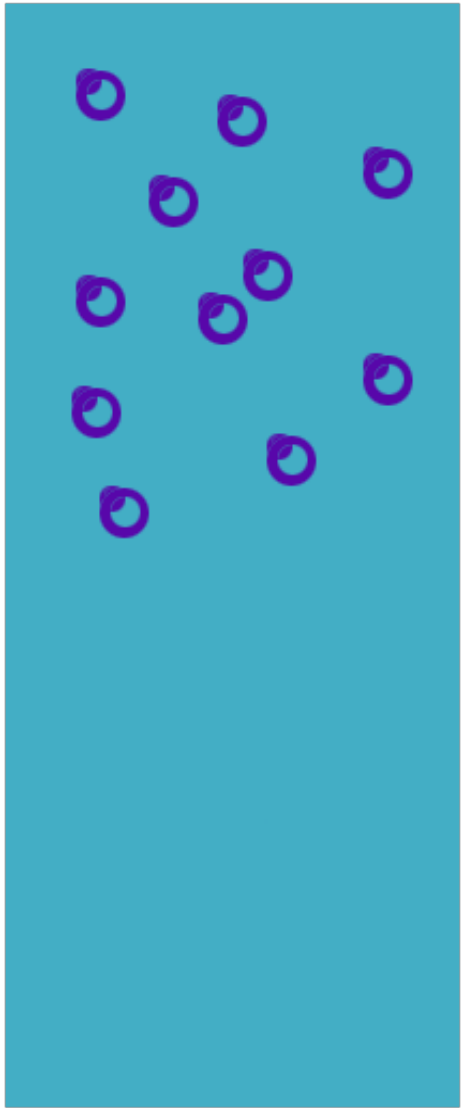
Thin & Thick Blood Smears

Thin





Thick

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Best Practice 7: Checklist Requirement

MIC.52200	**Thick and Thin Films**	**Phase II**
	Both thick and thin films (routine blood films and/or buffy coat films), or methods of equivalent sensitivity, are made to provide thorough examination for blood parasites.	

Source 1

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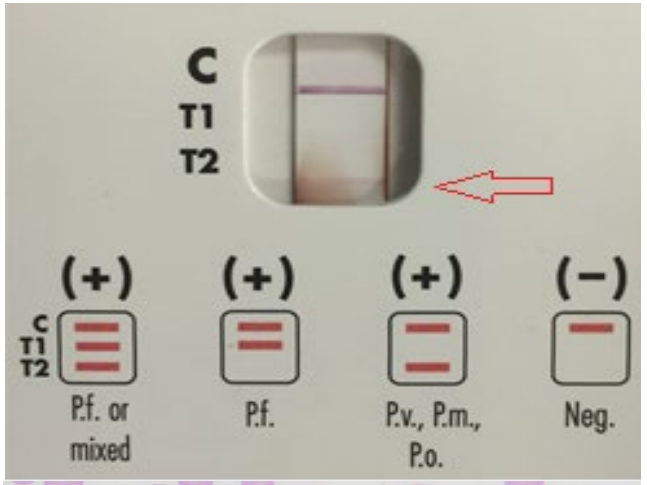
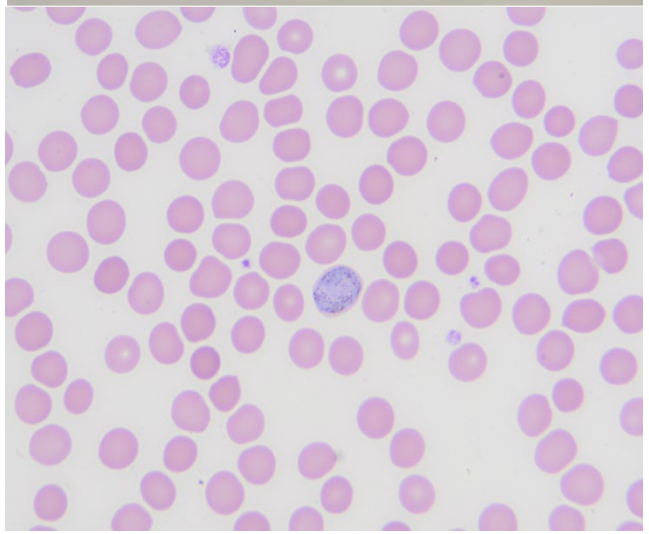
Best Practice 7: Malaria Detection

- While microscopic detection of malarial parasites is considered the gold standard for diagnosis, other assays are used to aid in rapid diagnosis
- Lateral flow immunochromatographic assay
  - Aids in identification of *P. falciparum* from other less virulent malaria species
  - Sensitivity depends on species, level of parasitemia, and prevalence
    - Sensitivity 95.5% for *P. falciparum*, 83.5% non-*falciparum* species (comparator method PCR)
  - Package insert states all negative results must be confirmed by microscopy





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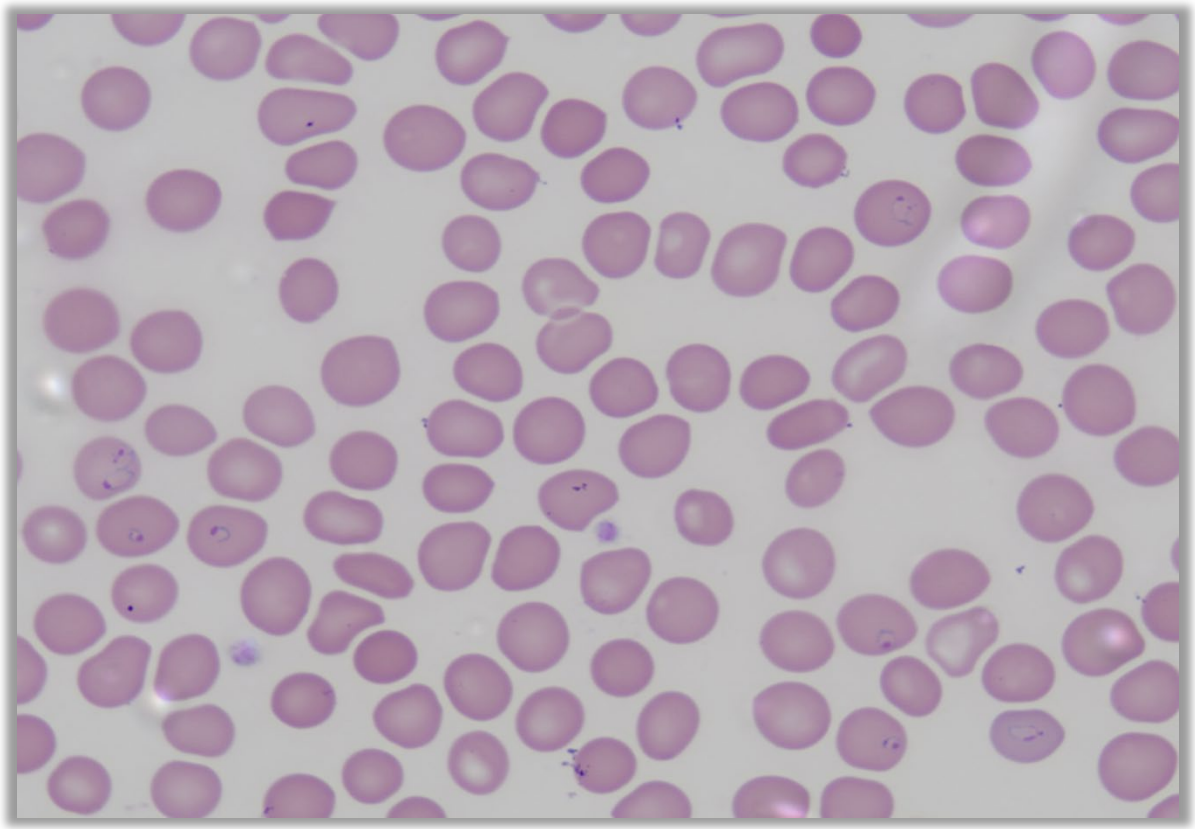
Best Practice 8: Adequate Review of Slides

- Both thick and thin slides should be thoroughly reviewed using oil immersion magnification to identify blood parasites
- Recommendation:
  - Approximately 100 fields for thick smears
  - Approximately 300 fields for thin smears



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Best Practice 8: Checklist Requirement

MIC.52260	Slide Review Procedure	Phase II
	An adequate number of fields is examined under the 100X oil immersion objective (eg, 300 fields).	

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Best Practice 9: Parasitemia Quantitation

- Parasitemia level is important as it guides proper selection of treatment and level of care, predicts prognosis, and assesses response to therapy
- Calculation from review of thin smear
  - Percent parasitemia (%) = (# of infected RBCs/total # of RBCs counted) x 100

Pro Tips

- Count at least 500 red cells, up to 2000 red cells when parasitemia is low
- Count multiply infected cells only once
- Don’t count gametocytes
- Be consistent in counting subsequent smears from same patient

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Best Practice 9: Checklist Requirement

MIC.52195 **Parasite Load Reporting** **Phase I**

When blood films are positive for malaria parasites (*Plasmodium* spp.), the parasite load (provided as percentage parasitemia or the number of parasites per µL of blood) is reported along with the organism identification.

NOTE: It is important to determine the parasite load when blood films are reviewed and found to be positive for malaria parasites because this information may be used to guide treatment decisions and monitor the response to therapy. Due to the potential for drug resistance in some of the Plasmodium species, particularly P. falciparum, it is important that every positive smear be assessed and the parasite load reported exactly the same way on follow-up specimens as on the initial specimen. This allows the parasite load to be monitored after therapy has been initiated. The parasite load will usually drop very quickly within the first 24 hours; however, in cases of drug resistance, the level may not decrease, but actually increase over time.

Although there are currently no requirements for reporting parasite load when blood films are positive for Babesia species, physicians may ask for these data to guide treatment decisions and monitor the response to therapy.

Evidence of Compliance:

✓ Patent reports for positive malaria cases

Source 1

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Proficiency Testing/External Quality Assessment Product Options

- Selection of PT/EQA products should reflect all testing that is performed for diagnostic purposes
- Testing, interpretation, and results reporting should be performed in the same manner as patient testing
- Remember no referral to reference laboratory

Parasitology P, P3, P4, P5

Procedure	Challenges per Shipment			
	Program Code			
	P	P3	P4	P5
Fecal suspension (wet mount)	2	5	2	
Fecal suspension (*Giardia* and *Cryptosporidium* immunoassays and/or modified acid-fast stain)	2	1	1	5
Giemsa-stained blood smear	1			
Preserved slide (for permanent stain)	2		3	

Additional Information

- The proficiency testing materials used for the Parasitology programs contain formalin as a preservative.
- Modified acid-fast stain results do not meet CLIA requirements for parasite identification.
- Number of specimen types are indicated in chart.

Blood Parasite BP

Procedure	Program Code	Challenges per Shipment
	BP	
Blood parasite identification (thin/thick film sets*)	▮	5

*This program will include corresponding thick films when available.

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Citations

- **Source 1:** College of American Pathologist Checklist, October 24, 2022 edition
- **Source 2:** Image source: CDC, Global Health, Division of Parasitic Diseases and Malaria. DPDx: Giardiasis. https://www.cdc.gov/dpdx/giardiasis/index.html Reuse of material does not imply endorsement by the CDC.
- **Source 3:** Image sources: Left: CDC, Global Health, Division of Parasitic Diseases and Malaria. DPDx: Diphyllobothriasis. https://www.cdc.gov/dpdx/diphyllobothriasis/index.html ; Right: CDC, Global Health, Division of Parasitic Diseases and Malaria. DPDx: Fascioliasis. https://www.cdc.gov/dpdx/diphyllobothriasis/index.html Reuse of material does not imply endorsement by the CDC.
- **Source 4:** Leber AL. Clinical Microbiology Procedures Handbook, 4th Edition. Washington, DC ASM Press; 2016.
- **Source 5**: Francis E. G. Cox , Gary P. Wormser, Atlas of Human Parasitology, 5th Edition By Lawrence R. Ash and Thomas C. Orihel Chicago, IL: American Society for Clinical Pathology Press, 2007 Clinical Infectious Diseases, Volume 45, Issue 9, 1 November 2007.
- **Source 6:** CDC - DPDx Homepage. Published 2019. https://www.cdc.gov/dpdx/index.html
- **Source 7:** Image Courtesy of courtesy of Missouri State Public Health Laboratory.

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Citations continued

- **Source 8:** Image Courtesy of Dr. Munaf Desai, AL Qassini Hospital, Shatjab, UAE.
- **Source 9:** Image source: CDC, Global Health, Division of Parasitic Diseases and Malaria. DPDx: Amebiasis. https://www.cdc.gov/dpdx/amebiasis/index.html Reuse of material does not imply endorsement by the CDC.
- **Source 10:** Image source: CDC, Global Health, Division of Parasitic Diseases and Malaria. DPDx: Intestinal (Non-Pathogenic) Amebae. https://www.cdc.gov/dpdx/intestinalamebae/index.html Reuse of material does not imply endorsement by the CDC.
- **Source 11:** Image source: CDC, Global Health, Division of Parasitic Diseases and Malaria. DPDx: Blastocystis sp. https://www.cdc.gov/dpdx/intestinalamebae/index.html Reuse of material does not imply endorsement by the CDC.
- **Source 12:** Prevention CC for DC and. CDC - Malaria - Travelers - Malaria Information and Prophylaxis, by Country. www.cdc.gov. Published June 21, 2019. Accessed June 4, 2023. https://www.cdc.gov/malaria/travelers/country_table/i.html
- **Source 13:** Image source: CDC, Global Health, Division of Parasitic Diseases and Malaria. DPDx: Lymphatic Filariasis. https://www.cdc.gov/dpdx/lymphaticfilariasis/index.html Reuse of material does not imply endorsement by the CDC.

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