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Diagnostic Medical Parasitology

FAQ's

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Contents

I. STOOL PARASITOLOGY
Commonly Asked Questions About Stool Parasitology

As laboratory personnel become more widely cross-trained, the availability of people who have expertise in diagnostic parasitology will become more limited. Over the years, many questions have been asked regarding various aspects of diagnostic medical parasitology. Answers to these questions may be helpful for those working in this area of microbiology and may provide some "tips of the trade" that are learned through many years of bench experience.

Specimen Collection
Intestinal Tract

1. **What has been the "gold standard" for stool collection systems?**
   The standard two-vial collection set has been used for many years and consists of one vial of 10% formalin from which the concentration is performed and one vial of liquid fixative [base fixative can be mercury (Schaudinn's fixative) or non-mercury to which has been added the plastic resin powder – polyvinyl alcohol, PVA (stool adhesive)] from which the permanent stained smear is prepared.

2. **What is the basis for the recommendation that three stools should be collected on alternate days, rather than three days in a row or three in one day?**
   Intestinal protozoa are shed in the stool on a cyclic basis. It is generally accepted that this time frame is approximately 10 days, so if specimens are collected too close together, sampling may occur at a low point in the cycle and the organisms may be missed. By collecting specimens over a wider time frame, days with heavier shedding may be sampled, as well.

3. **Can the laboratory accept three stools collected three days in a row?**
   Yes, but chances of sampling during the total time frame for shedding (10 days) might be more likely to include days of heavy shedding.

4. **Is it ever appropriate to accept three stools in one day from the same patient?**
   Generally, the answer is no with rare exceptions. If a patient has severe diarrhea or dysentery, there is a large dilution factor that may make finding any organisms present more difficult. Therefore, after consultation with the physician, more than one stool could be examined within any one day.

5. **Is it good laboratory practice to accept 2 (rather than 3) stools for the O&P examination?**
   Although the examination of 3 stool specimens provides a more statistically accurate result, many laboratories feel the percentage of organism recovery with the examination of 2 stools is acceptable. Percent recovery varies from ~80% to over 95% with the submission and examination of two stool specimens from a single patient. The examination of a single stool specimen is only about 50% accurate in the detection of parasites, especially intestinal protozoa. In one study, they found the following increases examining the third stool: **Hiatt, et al. (Am. J. Trop. Med. Hyg. 53:36-9, 1995)**
   - **Yield increased 22.7%: *Entamoeba histolytica***
   - **Yield increased 11.3%: *Giardia lamblia***
   - **Yield increased 31.1%: *Dientamoeba fragilis***

6. **Do you have to collect the stool specimen any differently if you are looking for the coccidia (*Cryptosporidium* spp., *Cyclospora cayetanensis*, or *Isospora (Cystoisospora) belli*)?**
   Fresh stool or specimens preserved in routine stool fixatives (formalin-based) can be used for diagnostic procedures for the identification of the coccidial oocysts (special modified acid-fast stains for the coccidia or fecal immunoassays for *Cryptosporidium* spp.). Some of the single vial collection options (Universal Fixatives) are also acceptable.

7. **Do you have to collect the stool specimen any differently if you are looking for microsporidia?**
   Fresh stool or specimens preserved in routine stool fixatives (formalin-based) can be used for diagnostic procedures (Modified Trichrome stains) for the identification of the microsporidial spores. Some of the single vial collection options (Universal Fixatives) are also acceptable.

Fixatives And Adhesives

1. What are the pros and cons of using stool fixatives?
   Although it might be helpful to see motile organisms in the direct saline wet preparation, often the trophozoite forms have already begun to disintegrate by the time the stool specimen reaches the laboratory. **Trophozoites DO NOT ENCYST after the stool has left the body**. In order to reduce the lag time between passage of the specimen and submission/arrival in the laboratory, stool fixatives are recommended. **The benefits of fixation and preservation of organism morphology far outweigh the benefits of receiving a fresh, unpreserved stool.**

2. **When selecting a single vial stool fixative, what questions should be asked?**
   Can one perform a concentration, permanent stained smear, special stains for the coccidia and microsporidia, and/or fecal immunoassay procedures from the specimen received in that vial (Universal Fixative)? What stains work best with that particular fixative? Another issue for consideration involves the types and numbers of parasites that might be missed using this approach.

3. **What are the advantages and disadvantages of using 5% vrs. 10% formalin?**
   Although 5% formalin is thought to provide a more "gentle" fixation of protozoa, this concentration formalin will not always kill all helminth eggs. The 10% formalin will kill most helminth eggs (exception: *Ascaris lumbricoides*), but may be more damaging to protozoa. In reality, most people probably can't tell the difference by examining clinical specimens preserved in either 5% or 10% formalin.

4. **What is the difference between buffered and nonbuffered formalin?**
   Buffered formalin tends to produce fewer osmotic changes in the organisms during fixation; however, on a day-to-day basis, it may be difficult to detect morphologic differences from buffered or unbuffered formalin.

5. **Should the laboratory be concerned about the amount of formalin used within the parasitology lab? Also see a complete discussion under the Concentration Section.**
   The amount of formalin used in diagnostic parasitology testing is very minimal. The regulations governing formalin use were developed for industry, where large amounts of formalin are sometimes used. However, the regulations do indicate that any place using formalin must be monitored. Once you have been monitored and results do not exceed the stated limits and your records are on file, there is no need to be rechecked again

(unless something dramatically changes in terms of your formalin volume use). **We have never heard of any microbiology laboratory coming even close to the limits, so formalin use within diagnostic parasitology is perfectly acceptable.**

6. **What is PVA (stool adhesive)?**
   PVA stands for polyvinyl alcohol, a plastic powder/resin that is incorporated into the liquid fixative (Schaudinn's or other fixatives) and serves as an adhesive to "glue" the stool material onto the slide. **PVA itself has no preservation capability and is inert in terms of fixation.**

7. **What are some good stool fixative/stain combinations?**
   The "gold standard" has been 1 vial of 10% formalin, from which the concentration is performed. The second vial has been with mercuric-chloride liquid fixative to which has been added PVA, from which the permanent stained smear is prepared and then stained (trichrome or iron hematoxylin). Other options in terms of overall quality are:
   a. SAF and iron hematoxylin stain
   b. UNIFIX, Z-PV, or TOTAL-FIX (Medical Chemical Corporation, Torrance, CA) and trichrome stain
   c. ECOFIX and ECOSTAIN (Meridian Biosciences)
   d. SAF and trichrome stain (not as good as SAF and iron hematoxylin)

8. **What are Universal Fixatives?**
   Examples of "Universal Fixatives" are: SAF (no mercury or PVA; contains formalin), TOTAL-FIX (no mercury, PVA or formalin), and ECOFIX (no mercury of formalin; contains PVA). **Currently, TOTAL-FIX is the only fixative that contains NO formalin, NO PVA, or NO mercury. TOTAL-FIX can be used without the addition of the PVA adhesive to the fixative – adequate drying time for smears prior to staining is the most important step (minimum of 1 hr in 37ºC incubator – requires more time for thicker fecal smears).**

Specimen Processing

Ova and Parasite Examination

1. **What procedures constitute the Ova and Parasite (O&P) Examination?**
   The direct wet smear, concentration, and permanent stained smear constitute the routine O&P examination on fresh stool specimens. **If the specimens are submitted to the laboratory in stool preservatives, then the concentration and permanent stained smear should be performed.** If a laboratory indicates they provide an O&P examination, the CAP checklist indicates that the O&P examination must include the concentration and permanent stained smear. Also, the CAP checklist requires the direct wet smear be performed on FRESH liquid or soft stool only (looking for motile trophozoites). There is no need to perform a direct wet mount on fresh formed stool; the possibility of seeing motile trophozoites is rare, since formed stool tends to contain only the cyst forms.

2. **Why do you need to perform a permanent stained smear examination on every stool submitted for an O&P examination?**
   There is data to indicate that intestinal protozoa may or may not always be seen and identified from the concentration examination. **Since the permanent stained smear is designed to facilitate identification and/or confirmation of the intestinal protozoa, it is important that this procedure be performed on all stools for which the O&P has been ordered.** Also, since trophozoite stages will not be visible on the concentration examination (rare exceptions - trophozoites can be seen in concentration sediments prepared from SAF-preserved specimens), it is even more important to examine the permanent stained smear. Even if organisms (trophozoites and/or cysts) are seen in the concentration wet mount, they might not be identified accurately and will require confirmation from the examination of the permanent stained smear. Remember, in patients who are symptomatic with diarrhea, they are more likely to have protozoan trophozoites in the stool, not the more resistant cyst form. This approach is consistent with the O&P examination (CAP Checklist for laboratory inspections).

3. **Why do you need to pour out some PVA onto paper towels prior to preparation of slides for permanent staining?**
   The reason this step has been included in processing directions is the following: Many people were taking the PVA/stool right out of the vial and onto the slide – too much PVA was being carried over onto the slide. It takes quite a bit of time for PVA to dry, thus the material often falls off during staining because the thick PVA is not yet dry. **Also, the amount of PVA (plastic powder) it takes to glue the stool onto the slide is extremely small.** So, if you eliminate the excess PVA prior to making the smears, your slides will require less drying time, the stool will adhere to the glass, and you will get a better stained smear. However, if the material (PVA/stool mixture) is taken right out of the vial onto the glass slide and this approach is working (no excess PVA) – there is no need to change protocols.
4. **Can you use concentrated sediment to prepare slides for permanent staining?**
   The main thing to remember is that a routine permanent stain (Ex: trichrome stain) cannot be performed from the concentration sediment if the specimen was originally preserved in formalin, or has been rinsed using formalin, saline, or water. One can perform a trichrome stain on SAF-preserved material, but not if the specimen has been rinsed with formalin, saline, or water. If you centrifuge the fecal/fixative material (without adding any rinse reagents), then providing your fixative is compatible with the permanent stain you are using, you can use some of the sediment for smear preparation prior to staining. However, once you continue with the rinse steps, the final concentration sediment will often (again depending on the rinse fluids) not be compatible with trichrome staining. **If you want to use a single vial fixative system (Universal Fixative), then you can spin down the stool/fixative mixture (using no additional rinse reagents), prepare your slide for permanent staining from the sediment, then proceed with the regular rinses called for in the concentration procedure.**

Diagnostic Methods

Direct Wet Examinations

1. **What is the purpose of the direct wet examination?**
   This procedure is designed to allow the viewer to detect motile trophozoites; this procedure should not be performed on preserved specimens and should be reserved for fresh stool specimens that are very soft or liquid. Often, the organism identification will be presumptive; permanent stained smears will also need to be examined.

2. **How should the direct wet preparation be examined?**
   The entire coverslip preparation (22 x 22mm) should be examined under low magnification (X100, 10X objective); approximately 1/3 to 1/2 of the coverslip preparation should be examined under high dry magnification (X400, 40X objective). **It is not practical to examine this preparation using**

oil immersion magnification (X1000, 100X oil immersion objective). Saline and/or iodine mounts can be examined; however, iodine will kill any organisms present, so trophozoite motility will no longer be visible.

3. **What do you expect to see during a wet preparation examination?**
Helminth eggs and/or larvae can be seen, as well as some protozoan cysts, WBCs, some yeast, and fecal debris. Many of the intestinal protozoa will need to be confirmed using the oil immersion magnification (X1000, 100X oil immersion objective) for the permanent stained smear.

Concentrations

1. **What is the recommended time and speed for centrifugation for the concentration method? Why is this important?**
The current recommendation is for every centrifugation step in the concentration method (sedimentation) to be performed for **10 min at 500 Xg**. If this recommendation is not followed, then small coccidian oocysts and microsporidial spores may not be recovered in the concentrate sediment. The number of organisms is greatly increased over taking the sample from the unspun specimen.

2. **What is the purpose of the concentration procedure?**
The purpose of the concentration is to concentrate the parasites present, either through sedimentation or flotation. The concentration is specifically designed to allow recovery of protozoan cysts, coccidian oocysts, microsporidian spores and helminth eggs and larvae.

3. **Why is the flotation concentration used less frequently than the sedimentation concentration?**
There are several reasons. First, **not all parasites will float; therefore, you need to examine both the surface film and the sediment before indicating the concentration examination is negative.** Second, the organisms must not be left in contact with the high specific gravity zinc sulfate for too long or protozoa will tend to become distorted, so the timing of the examination is more critical. Also, the specific gravity of the fluid will need to be checked periodically.

4. **What specific gravity zinc sulfate should be used for the flotation concentration procedure?**
If the concentration is being performed on fresh stool, the specific gravity of the solution should be 1.18. However, if the concentration is being performed on stool preserved in a formalin-based fixative, the specific gravity of the zinc sulfate should be 1.20.

5. **How should the concentration wet preparation be examined?**
Formalin-ethyl acetate (substitute for ether) sedimentation concentration is the most commonly used. Zinc sulfate flotation will not detect operculated or heavy eggs (Ex: unfertilized *Ascaris* eggs); both the surface film and sediment will need to be examined before reporting a negative result. Smears prepared from concentrated stool are normally examined as for the direct wet mount using the low power objective (10X) and the high dry power objective (40X); use of the oil immersion objective (100X) is not recommended (organism morphology not that clear). The addition of too much iodine may obscure helminth eggs (will mimic debris).

6. **What semi-automated methods are available to read the concentration sediments?**
The traditional sampling approach using pipettes and the preparation of wet smears using glass slides and coverslips can be replaced with a semi-automated sampling and viewing system from DiaSys Corporation. The specimen is drawn through tubing from the mixed concentrated stool sediment into two viewing chambers that fit onto the microscope stage. The quality of the glass is excellent and organism morphology can be easily seen within the viewing chambers. Selection of such a system often depends on the laboratory work load.

7. **Are there any tips for specimen processing for detection of the microsporidia?**
When early comparisons were performed on methods, the authors used a slower centrifugation compared with using unspun material. Using these methods, they felt using unspun material was better for recovery of microsporidial spores. **However, at UCLA when we looked carefully at this approach <u>compared with the 500 Xg for 10 min (standard centrifugation time and speed), we found considerably more spores in the sediment.</u>** If the stool contains a lot of mucus or is runny, just add formalin and centrifuge. Don't bother with ethyl acetate since it may pull much of the material you want to examine up into the mucus layer. If the stool is not particularly watery or doesn't contain a lot of mucus, you can treat it just like a regular concentration (but remembering to make every spin at 500 Xg for 10 min). Also, the more you manipulate a stool, the more likely you will lose some organisms (applies to all parasites in stool). So, you may want to eliminate the wash steps and work with the first sediment you obtain from the first spin.
Remember to make the smears pretty thin - this will help you see the spores, but also remember not to decolorize too much. Filtration is not a problem using any of the commercial concentration systems. If you are using gauze, make sure you use woven gauze and use only two layers (do not use pressed gauze, which is too dense and will trap parasites)**.**

8. **Comment on the use of formalin within the microbiology laboratory.**
The formalin regulations were originally developed for industry (plywood, etc.) where great amounts of formalin are used in the manufacturing process. The amount of formalin we are exposed to in the laboratory is very minimal; we've never heard of any microbiology laboratory (including a full-service parasitology service) even coming close to the limits.

"The Occupational Safety and Health Administration (OSHA) amended the original regulations for occupational exposure to formaldehyde in May of 1992 (1:Fed Regist 1992 May 27;57(102):22290-328). The final amendments lower the permissible exposure level for formaldehyde from 1 ppm (part per million) as an 8-hour time-weighted average (TWA) to an 8-hour time-weighted average of 0.75 ppm. The amendments also add medical removal protection provisions to supplement the existing medical surveillance requirements for those employees suffering significant eye, nose or throat irritation and for those suffering from dermal irritation or sensitization from occupational exposure to formaldehyde. In addition, changes have been made to the standard's hazard communication and employee training requirements. These amendments establish specific hazard labeling for all forms of formaldehyde, including mixtures and solutions composed of 0.1% or greater of formaldehyde in excess of 0.1 ppm. Additional hazard labeling, including a warning that formaldehyde presents a po-

tential cancer hazard, is required where formaldehyde levels, under reasonably foreseeable conditions of use, may potentially exceed 0.5 ppm. The final amendments also provide for annual training of all employees exposed to formaldehyde at levels of 0.1 ppm or higher."

Those laboratories that have been monitored have not come close to either measurement. Once a laboratory has been measured and the results (below thresholds for regulatory requirements) are on file, this information does not need to be generated again. No badges are required. Even without a fume hood (many labs do not use a fume hood), performing the routine formalin-ethyl acetate concentration does not seem to be a problem. A number of people who have indicated they want to remove formalin from the laboratory probably don't really understand the history of the regulation or the actual issues. The only possible problem seen in the clinical laboratory/pathology setting might be a routine anatomical pathology laboratory where very large amounts of formalin were used, and with sloppy use. However, even within large microbiology laboratories, it does not seem to be a problem.

Permanent stains

1. **What is the purpose of the permanent stained smear?**
   The purpose of the permanent stained smear is to provide contrasting colors for both the background debris and parasites present; it is designed to allow examination and recognition of detailed organism morphology under oil immersion examination (100X oil immersion objective for a total magnification of X1000). This examination is primarily designed to allow recovery and identification of the intestinal protozoa.

2. **How long should the permanent stained smear be examined?**
   Rather than responding with a specific number of minutes, the recommendation is to examine at least 300 oil immersion (X1000 total magnification) fields; additional fields may be required if suspect organisms have been seen in the wet preparations from the concentrated specimen.

3. **What recent changes have influenced the overall quality of the permanent stained smear?**
   The use of mercury substitutes in fecal fixatives generally leads to diminished overall morphology quality of the intestinal protozoa. However, some of the mercury substitutes (zinc-based) provide morphologic quality that is close to mercury and allows identification of most of the intestinal protozoa. Differences in detection and identification are usually comparable unless there are very few organisms present or the organisms are quite small. In these circumstances, some organisms may be missed using mercury substitutes. The development of the Universal Fixative, TOTAL-FIX (no mercury, no formalin, no PVA) provides a fixative that results in excellent overall protozoan morphology.

4. **What is the purpose of the iodine dish in Wheatley's trichrome stain protocol?**
   Mercury is removed from the smear when placed in the iodine dish; there is a chemical substitution of iodine for mercury. The iodine is removed during the next two alcohol rinses. Therefore, when the slide is placed in trichrome stain, neither mercury nor iodine remains on the smear.

5. **Why don't you need to use the iodine dish when staining fecal smears prepared from specimens preserved in the newer single vial systems (zinc sulfate based fixative)?**
   The zinc sulfate-based fixative is water soluble, so the dry smears can be placed directly into the trichrome dish without having to go through the iodine and subsequent rinse steps. The zinc sulfate will be removed by the water in the trichrome stain.

6. **Why might you have to use the iodine dish and subsequent rinses in your staining set up when staining slides from the proficiency testing agencies (AAB, various states, etc.)?**
   Some smears used for proficiency testing are prepared from fecal specimens that have been preserved in mercury-based fixatives, so the iodine dish and subsequent rinse steps are required to remove mercury and iodine prior to staining with either trichrome or iron hematoxylin stains. For several years, CAP proficiency testing specimens have been preserved in non-mercury fixatives; thus the iodine dish is not required.

7. **What role does the acetic acid play in the trichrome stain?**
   Both the trichrome and iron hematoxylin stains are considered regressive stains; the fecal smears are overstained and then destained. The acetic acid in the 90% alcohol rinse step in the trichrome stain removes some of the stain and provides better contrast. However, in some cases differences in the quality of staining between stained protozoa that have been subjected to the 90% alcohol rinse with and without the acetic acid may be difficult to detect. It is important not to destain the smears too much; overall organism morphology will be diminished.

8. **What causes the xylene (or xylene substitute) dehydration solutions to turn cloudy when a slide from the previous alcohol dish is moved forward into the xylene dish?**
   If there is too much water carryover from the last alcohol dish, the xylene solution may turn cloudy. When this occurs, replace the 100% alcohol dishes, back up the slide into 70% alcohol (you can also use a series of steps - 95%, then 70%), allow it to stand for 15 min and then move the slide forward through the 100% alcohol steps and xylene steps.

9. **Why is absolute ethanol (100%) recommended as the best approach?**
   Although many laboratories use the commercially available 95/5% denatured alcohol mix as their "absolute alcohol", the dehydration of stained fecal smears will not be as good as that obtained with 100% ethanol. You may want to add an additional dish of the denatured alcohol (absolute alcohol) to your staining setup to obtain better dehydration.

10. **What is the difference between xylene and xylene substitutes?**
    There are several differences. Most laboratories have made the decision to eliminate xylene from their laboratories as a safety measure. However, xylene substitutes generally do not dehydrate as well. Also, after the slides are removed from the last dish of xylene substitute, they take longer to dry. You may want to add an additional dish of the xylene substitute to your staining setup to obtain better overall dehydration.

Stool Immunoassay Options

1. **What are some of the immunoassay options available for stool protozoa?**
   Currently, there are immunoassays available for *Giardia lamblia, Cryptosporidium* spp., the *Entamoeba histolytica/E. dispar* group, and *Entamoeba histolytica*. Reagents for the detection of *Dientamoeba fragilis, Blastocystis hominis,* and the microsporidia are under development.

2. **What methods are available commercially?**
   Direct fluorescence (DFA), enzyme immunoassay (EIA), and cartridge formats (membrane flow or a solid phase qualitative immunochromatographic procedure) are currently available.

3. **Why might someone want to use a fecal immunoassay option?**
   If the most common organisms found in the area are *Giardia, Cryptosporidium* and/or the *Entamoeba histolytica/E. dispar* group, then fecal immunoassays are certainly options. Specific patients/history/symptoms would suggest the use of fecal immunoassays. **Both the O&P examinations and fecal immunoassays are recommended for a laboratory test menu – both would be orderable, billable procedures.**

4. **How would stool immunoassay requests fit into a laboratory that also performs Ova and Parasite (O&P) examinations?**
   Any diagnostic laboratory performing routine parasitology testing should offer both options: routine O&P examinations and the fecal immunoassays. **Both the O&P examinations and fecal immunoassays are recommended for a laboratory test menu – both would be orderable, billable procedures.**

5. **What do you mean by the "routine O&P examination"?**
   This test includes (fresh material- liquid or semiliquid stools) a direct wet exam, the concentration and the permanent stain; preserved specimens would require a concentration and permanent stained smear (no direct wet mount is required).

6. **How would you fit the fecal immunoassays into your laboratory?**
   Per separate requests, you could offer the routine O&P examination and you could also offer on request the stool immunoassay option. Both options should be in the laboratory ordering test menu.

7. **What type of educational initiatives would have to be undertaken prior to offering these options?**
   Physicians would need to know the pros and cons of ordering either the immunoassay or the routine O&P examination. **Refer to the table on test ordering.**

8. **What are some of the pros and cons for the fecal immunoassays?**
   Pros:
   · Depending on the format selected, the immunoassays are fast and relatively simple to perform.
   · The result can "rule in or out" very specific organisms.
   · If the patient becomes asymptomatic at the time the immunoassay is negative, additional testing may not be necessary.
   · May help reduce personnel costs (time to perform procedures).
   · The fecal immunoassays are more sensitive than the routine O&P exams and/or the special stains for the coccidia or microsporidia.

   Cons:
   · The fecal immunoassay kits test **only** for selected organisms.
   · Depending on the format, they might be somewhat complex to perform.
   · Test requests may not justify certain formats (cost, equipment, or training).
   · It is critical that the physician realize that a negative immunoassay will not rule out all possible parasitic etiologic agents causing diarrhea.
   · **In the case of *Giardia lamblia*, it may require a fecal immunoassay on two different stool specimens in order to get a positive result. This is not the case for other protozoa.**

9. **Can the fecal immunoassays be used for duodenal fluid (giardiasis)?**
   First of all, the tests have NOT been approved or validated for this type of specimen. Also, the duodenal fluid/aspirate would contain primarily the trophozoite form, not the cyst stage for which the reagents have been designed. An example: if one is using the FA (rather than EIA or cartridge) the trophs may appear to be fluorescent at a very pale 1+, while the cysts are a strong 3-4+. So, while there may be a few antigenic sites that are shared by the cysts and trophs, the commercial tests for *Giardia* are detecting the cyst antigens.
   You can "try" the reagents on these specimens, but if the result is negative, the result in no way has "ruled giardiasis out." I would recommend testing stool only; if the result was positive, this approach might avoid the need for duodenal aspirate testing altogether.

10. **Comment on the use of the *Giardia* fecal immunoassay; how many specimens should be tested before assuming the patient is negative?**
    Since the evidence indicates that *Giardia* is shed sporadically, and that more than one immunoassay might be required to diagnose the infection, the recommendation is similar to that seen for stool collection for the routine O&P examination: **for *Giardia*, perform the immunoassay on two different stools (assuming the first specimen is negative)**, collected within no more than a 10 day period - a good collection schedule would be day one, then day three or four. That way, one would assume one of those collections would yield a positive if the organisms (in sufficient numbers) are present. However, you also have to remember that **if the patient is a carrier with a low organism load, even the second immunoassay might be negative. Note: One stool is sufficient for immunoassay testing for *Cryptosporidium* spp.**

11. **Although an FA for *Cryptosporidium/Giardia* was negative, the O&P concentrate showed *Giardia* trophs on the wet mount. Might we miss positive *Giardia* if only the FA is ordered? Do Also, do any of the EIA antigen tests detect both trophs and cysts and should we switch to one of those?**
    Although the antibody in the immunoassay kits is to the cyst antigen (primarily, but it is a polyclonal reagent), in some of the kits the trophs do fluoresce, but much less intense (around a 1+, maybe even a 2+, thus indicating some shared epitopes). In almost all patients, there will be a combination of cysts and trophs, unless the patient has active diarrhea and is passing only trophs (no time for cysts to form with rapid passage through the GI tract). However, most patients will have cysts, as well, and the results (if above the test limits for sensitivity) will be positive. The situation you describe can happen, but it's probably not that common. **However, with ONLY trophs present, you may get a negative result. Some of the kits**

tend to provide a bit higher fluorescence with the troph, but it varies.

12. **After the patient has been treated, how long will the *Giardia* antigen test remain positive?**
It has been recommended that you test about 7 days after therapy, hopefully to avoid picking up residual antigen. However, if you wait too long (several weeks), you always run the risk of picking up antigen from a possible reinfection. Some also feel that low antigen levels can be found for up to about 2 weeks. A good time frame for retesting would probably be about 7-10 days after therapy. If the first specimen at 7 days is still positive, then you could retest at 10-12 days. Also, we know that the immunoassays (for diagnosis) may not pick up low antigen loads (organism shedding issues), thus the recommendation for diagnostic immunoassay testing for giardiasis has been changed and recommends performing immunoassay testing on one additional stool specimen (if the first one is negative). The testing on two different stool specimens should be performed within about 3-5 days.

13. **Why do fecal immunoassay kits that test for either *Entamoeba histolytica* or the *Entamoeba histolytica/E. dispar* group require fresh or frozen stools or stools submitted in Cary-Blair?**
Unfortunately, at the present time, these reagents will not function properly on preserved fecal specimens. Although the manufacturers are trying to develop such kits, they are not yet available.

14. **How long will antigen survive in fresh stools?**
It is recommended that fresh stools be tested within 24 h of collection; they can be stored overnight in the refrigerator. They can also be frozen or preserved in 10% formalin prior to testing (both freezing and/or formalin preservation methods will preserve antigen for long periods of time – even years).

Organism Identification And Diagnostic Methods

Protozoa

1. **What is the most effective technique for the identification of the intestinal protozoa?**
Although some protozoan cysts can be seen and identified on the wet preparation smear (direct mount, concentration sediment wet mount), the permanent stained smear is the procedure that is recommended as the most relevant and accurate for the identification of the intestinal protozoa. These preparations are examined using the oil immersion objective (100X) for a total magnification of X1000. At least 300 oil immersion fields should be examined prior to reporting the permanent stained smear result.

2. **Are trophozoites ever seen in the wet mounts of stool?**
Usually the trophozoites are not seen in the concentration sediment wet mount preparations unless they are prepared from stool preserved in Universal Fixatives. Motile trophozoites can occasionally be seen in the direct wet smear, but the number of times this occurs is rare.

3. **What are some of the tips to consider when reporting *Entamoeba hartmanni*?**
The measurements of 10 microns or less for the *Entamoeba hartmanni* cyst refer to wet preparation measurements, so the measurement should be decreased ~1 micron on the permanent stained smear. When you see a cyst on the permanent stained smear, there is often a halo representing shrinkage. **The cyst needs to be measured to include that halo.** On the bench, the measurements for this cyst generally run from around 9.5 down to about 8 microns and they are morphologically definitely *E. hartmanni*. The *E. hartmanni* cyst generally contains more chromatoidal bars than are seen in *E. histolytica/E. dispar*. Also the *E. histolytica/E. dispar* cysts tend to measure routinely on the bench (trichrome slides) from about 10.5 up to about 13 or so. Also, on the bench, the *E. hartmanni* nuclei, particularly in the troph, will tend to look like a "bulls eye" - very sharp line of nuclear chromatin with the karyosome right in the middle.

4. **Do nonpathogenic protozoa ever cause symptoms?**
Endolimax nana, Iodamoeba bütschlii, Chilomastix mesnili, and *Pentatrichomonas hominis* (as examples) have been categorized as nonpathogens. Although rare, patients have been documented who have been symptomatic with a nonpathogen. However, it is sometimes difficult to determine from the case history the extent of the workup, including coccidia and the microsporidia. Before assigning symptoms to nonpathogenic protozoa, a comprehensive search for other proven pathogens should be performed.

5. **What color is the autofluorescence seen with *Cyclospora cayetanensis*?**
The color depends on the particular FA filters used. If the filters are used for Calcofluor white, the oocysts will appear as pale blue rings; if the filters are used for FITC, the oocysts will appear to be more yellow-green. Fluorescence intensity will vary from about 1+ to 2+; it is rare to see stronger autofluorescence with these oocysts.

6. **Why is *Blastocystis hominis* so controversial regarding pathogenicity?**
What we currently call ***Blastocystis hominis*** (morphologically) appears to be approximately 10 different strains or species, some of which are pathogenic and some are nonpathogenic. Based on continuing molecular studies, changes in the classification of this group could change soon. If all strains or species currently make up what is called "*Blastocystis hominis*" – this could explain the controversy regarding pathogenicity and the fact that some patients are symptomatic, while some are asymptomatic. Unfortunately, all of the strains or species are morphologically identical; therefore, the correct identification reported should remain *Blastocystis hominis*, and the number should be quantitated from the permanent stained smear (rare, few, moderate, many, packed).

Helminths

1. **Why can't all helminth eggs be recovered using the flotation concentration rather than the sedimentation concentration?**
Some helminth eggs are quite heavy (unfertilized *Ascaris* eggs) and will not float, even using zinc sulfate with a specific gravity of 1.20. Other helminth eggs are operculated; when the egg is placed in a high specific gravity solution, the operculum "pops" open and the egg fills with fluid and sinks to the bottom of the tube. Thus, both the surface film and the sediment should be examined before reporting the specimen as negative.

2. **Why do helminth larvae need to be identified to the species level; shouldn't all larvae recovered in stool be that of *Strongyloides stercoralis*?**
Although helminth larvae in stool are normally *Strongyloides stercoralis*, there is always the possibility that in fresh stool hookworm eggs have continued to mature and may hatch before the stool is processed and/or placed in fixatives. It is

important to make sure that the larvae seen are, in fact, the rhabditiform (non-infectious) larvae of *S. stercoralis* rather than larvae of hookworm. The agar plate culture is the most sensitive method for the recovery of *S. stercoralis* larvae. Also, remember that migrating larvae could also be recovered from respiratory specimens (sputum, BAL, etc.).

3. **Are there any specific recommendations for the detection of schistosome eggs?**
When trying to diagnose schistosomiasis, regardless of the species suspected you should be examining both stool (several different stool specimens) and urine (spot urines plus 24 hour specimen) (collected with no preservatives). Occasionally adult worms get into blood vessels where they aren't normally found (Example: *S. mansoni* eggs found in urine only). When you perform a sedimentation concentration, use saline to prevent premature egg hatching. Once you are ready to try a hatching procedure, then you can put the sediment into spring water (dechlorinated water) to stimulate hatching. When examining wet mounts under the microscope, you need to look for the movement of cilia on the larvae within the egg shell (thus the need to collect the specimens with no preservatives). You want to be able to tell the physician whether the eggs, if present, are viable or all you see are dead egg shells. If you suspect schistosomiasis, it is recommended that you examine a number of wet mounts, particularly if you aren't going to perform a hatching test.

4. **What is the most sensitive test for the diagnosis of strongyloidiasis?**
Agar plate cultures are recommended for the recovery of *S. stercoralis* larvae and tend to be more sensitive than some of the other diagnostic methods such as the O&P examination. Stool is placed onto agar plates and the plates are sealed to prevent accidental infections and held for two days at room temperature. As the larvae crawl over the agar, they carry bacteria with them, thus creating visible tracks over the agar. The plates are examined under the microscope for confirmation of larvae, the surface of the agar is then washed with 10% formalin, and final confirmation of larval identification is made via wet examination of the sediment from the formalin washings. See Section 5.

In a study looking at the prevalence of *S. stercoralis* in three areas of Brazil, the diagnostic efficacy of the agar plate culture method was as high as 93.9% compared to only 28.5% and 26.5% by the Harada-Mori filter paper culture and fecal concentration methods, when fecal specimens were processed using all three methods. Among the 49 positive samples, about 60% were confirmed as positive only using the agar plate method. These results indicate that the agar plate approach is probably a much more sensitive diagnostic method and is recommended for the diagnosis of strongyloidiasis.

It is important to remember that more than half of *S. stercoralis*-infected individuals tend to have low-level infections. The agar plate method continues to be documented as a more sensitive method that the usual direct smear or fecal concentration methods. Daily search for furrows on agar plates for up to 6 consecutive days results in increased sensitivity for diagnosis of both *S. stercoralis* and hookworm infections. Also, a careful search for *S. stercoralis* should be made in all patients with comparable clinical findings before deciding on a diagnosis of idiopathic eosinophilic colitis, because consequent steroid treatment may have a fatal outcome by inducing widespread dissemination of the parasite.

5. **Where can one get serologies for a *Baylisascaris procyonis* infection?**
Dr. Kevin Kazacos
1243 Veterinary Pathology Bldg
Purdue University
West Lafayette IN 47907-1243
Phone: 765 494-7556
Fax: 765 494-9830
Email: kkazacos@vet.purdue.edu

Reporting
Organism Identification

1. **Should common or scientific names be used when reporting the presence of parasites?**
The scientific name (genus, species) names should be used on the final report that goes to the physician and to the patient's chart. It is also recommended that the stage of the organism be included (trophozoite, cyst, oocyst, spore, egg, larvae, and adult worm - various stages of the malaria parasites/will impact therapy).

2. **What happens if there are several different names used in the literature (*Giardia lamblia, G. intestinalis, G. duodenalis*)?**
It is appropriate to use the most commonly accepted name (*Giardia lamblia*); you can also let the proficiency testing organism lists be your guide. When a replacement name begins to appear on the proficiency test list, then it's appropriate to notify your clients and institute the name change. Remember, it is very important to notify all clients prior to making any name changes.

3. **How has the reporting of Cryptosporidium parvum changed and why?**
It is now known that more than one species of *Cryptosporidium* can cause disease in humans (*C. parvum*: humans and other mammals; *C. hominis*: humans). However, the different species cannot be differentiated on the basis of morphology. It is now recommended that the more correct reporting format would be: ***Cryptosporidium*** spp. (rather than ***Cryptosporidium parvum***).

4. **Should WBCs and/or other cells or yeast be reported and why?**
The reporting and quantitation (rare, few, moderate, many) of WBCs (PMNs, macrophages, eosinophils) provides some additional information for the physician. If the patient continues to have diarrhea, it may give the physician something more to consider, particularly if stool culture has not been ordered. Also, conditions related to non-infectious diarrheas may also result in WBCs and macrophages in the stool. We always include this type of information/explanation in our educational process regarding reporting formats. Physicians can then decide on the relevance of the information. Remember, that when reporting WBCs, we can also identify eosinophils from the permanent stained smear (may or may not be related to parasitic infection and may or may not correlate with peripheral eosinophilia – this information may also be helpful.

Reporting yeast is a bit different. In order to report anything about yeast, you must know the stool was fresh or immediately put in preservative. If there are lots of yeast, budding yeast and/or pseudophyphae, then this provides some additional information for the physician, often depending on the patient's general condition and whether or not they are immunosuppressed. However, if you don't know whether the collection criteria were met, then reporting anything about yeast is NOT recommended since this type of report may be misleading.

5. **How should Blastocystis hominis be reported?**
In the past there was some agreement that the number of organisms present (mod, many, packed) were more likely to be associated with symptoms. However, in the past few years, there have been anecdotal reports of patients being symptomatic with rare or few organisms, as well. The current recommendation is to report the organism and quantitate per rare, few, mod, many, packed (using your proficiency testing quantitation scheme). It is important to confirm that the physicians know what the report means and understand the controversial issues surrounding this organism - they can then correlate the numbers with symptoms. If rare or few, there is no solid data per numbers relevance other than the anecdotal case reports (many of which are word of mouth). Many physicians are treating if the patient is symptomatic and no other organisms are found (including coccidia and/or microsporidia).

"*Blastocystis hominis*" includes several strains or species, some of which are pathogenic and some nonpathogenic. Based on continuing molecular studies, changes in the classification of this group could be updated. If all the strains or species make up what is morphologically called "*Blastocystis hominis*" – this could explain the controversy regarding pathogenicity and the fact that some patients are symptomatic, while some are asymptomatic. Unfortunately, strain differences can not be detected by morphology; they look alike.

6. **How should intestinal protozoa be reported?**
Reporting trophs/cysts: This has been the accepted way of reporting intestinal protozoa for a couple of reasons: Different drugs are used to treat *Entamoeba histolytica* cysts vs. trophs. Also, since the cyst form is the infective stage for the protozoa (exception: *D. fragilis* and the trichomonads), the report does convey some epidemiologic information. For these reasons, the current recommendation is to continue to report all protozoa (genus, species, and stage).

7. **What additional reporting comments should be added to the reporting of the O&P examination and the fecal immunoassays?**
<u>It is important to add the following comment to the O&P examination</u>:
"The O&P examination is not designed to detect the intestinal coccidia (*Cryptosporidium* spp., *Cyclospora cayetanensis*) or the microsporidia. *Isospora belli* oocysts (coccidia) can be detected from the concentration sediment examination."

<u>Report the results of the fecal immunoassays based on the specific organism(s) relevant to the kit:</u>
"No *Giardia lamblia* antigen detected." OR "*Giardia lamblia* antigen present."
"No *Giardia lamblia* or *Cryptosporidium* spp. antigen detected."
OR
"Negative for *Giardia lamblia*."
"Negative for *Giardia lamblia* and *Cryptosporidium* spp."

<u>The report of *Blastocystis hominis* should be accompanied by the following report comment:</u>
"*Blastocystis hominis* (morphologically) is comprised of a number of different strains or species, some of which are pathogenic and some are nonpathogenic. This explains why some patients are symptomatic and some are asymptomatic."

Quantitation

1. **What organisms should be quantitated in the final report to the physician and patient's chart?**
Organisms and non-organism cells/structures (stool blood cells, Charcot-Leyden crystals) that are recommended for quantitation include the following:
A. Intestinal protozoa: *Blastocystis hominis*
B. Helminths: *Trichuris trichiura* eggs, *Clonorchis sinensis* eggs, schistosome eggs (also report viability of *Schistosoma* spp. eggs).
C. Blood parasites: all malaria organisms and *Babesia* spp.
D. Blood cells (PMNs, macrophages, RBCs)
E. Charcot-Leyden (CL) crystals

2. **Why are all proficiency testing specimen answers reported and quantitated, while clinical specimen reports are rarely quantitated in terms of organism numbers?**

There are two issues to consider.
(1) When reporting proficiency testing (PT) specimen results, you are asked to quantitate organisms in both the formalin wet mounts and permanent stained smears. This information serves as a **quality control check for the proficiency testing agency to ensure that organism numbers are consistent throughout the challenge vials/slides**.

(2) Most laboratories (with few organism exceptions) do not quantitate organisms on the concentration or permanent stained smear reports. Some exceptions might be: *Trichuris trichiura* eggs in a concentration wet mount (light infections might not be treated). Since many organisms are shed on a random basis, quantitation may change dramatically from day to day and generally has little clinical relevance.

Proficiency Testing
Wet Preparations

1. **How should you examine a wet preparation for proficiency testing?**
Most directions will recommend you shake the vial and take a sample from the mixed vial contents. However, if the number of eggs is few, you may be better off taking a very small drop from the settled material. If you take too large a drop, it will be too thick to examine properly. Make sure you do not add too much iodine; very darkly stained helminth eggs can resemble debris.
2. **Are there any "tips" regarding the microscope set up for the examination of an unstained wet preparation?**
The microscope should be aligned properly using Köhler illumination. Make sure the light is not too bright; otherwise, you may shine right through the organisms and miss the parasites. Also, you may want to close the diaphragm a bit to provide a bit more contrast. Extra contrast is particularly important when reading with saline only (no iodine added).

Permanent Stained Smears

1. **How should you examine a permanent stained fecal smear for proficiency testing?**
Make sure the light is very bright (condenser all the way up) and the diaphragm is open. Review both thin and thick areas of the smear; examine at least 300 oil immersion fields (using the 100X oil immersion objective) before you report the specimen as a negative. If you use the 50X or 60X scanning oil immersion lens, you still need to review 300 oil fields as indicated above (using the 100X oil immersion objective).

II. TISSUE AND FLUIDS, PARASITOLOGY

Commonly Asked Questions About Tissues or Fluids

1. **How should tissues be submitted to the laboratory?**
   Tissue specimens should be immediately sent to the laboratory and kept moist during transit. If the tissue will be processed for culture (*Acanthamoeba, Naegleria, Leishmania* spp., *Trypanosoma* spp., or *Toxoplasma gondii*), the specimen should be kept sterile and submitted in a sterile container. If the tissue will be processed for wet examinations and permanent stained smears, any type of container is acceptable. Remember that the specimen must be kept moist; if it dries out in transit, neither culture nor stained smears will be acceptable.

2. How should eye specimens be submitted for ***Acanthamoeba*** culture?
   If sending the specimen in a tube/vial/ or screw-cap tube, make sure the container is filled with transport fluid (saline is acceptable). This will prevent the small tissue specimen from drying out if during transit it is shaken onto the side of the container. If the container is not full of fluid, the tissue may dry out and will be unacceptable for culture.

3. **How should duodenal drainage specimens be handled?**
   Duodenal drainage specimens must be submitted to the laboratory as quickly as possible for processing. Delays may prevent organism recovery and identification. These specimens may be very liquid and may contain a lot of mucus. It is best to centrifuge the tube, discard any supernatant fluid left, and examine the mucus only as wet preparations (low light) and/or permanent stained smears (trichrome, iron hematoxylin).

III. BLOOD PARASITOLOGY

Commonly Asked Questions Concerning Diagnostic Blood Parasitology

Many questions have been asked regarding various aspects of diagnostic medical parasitology and the examination of blood specimens. Answers to these questions may be helpful for those working in this area of microbiology and may provide some "tips of the trade" that are learned through many years of bench experience.

Specimen Collection
Blood

1. **If *Plasmodium falciparum* parasites are sequestered in the capillaries, why not do a finger stick, rather than a venipuncture?**
   The capillaries are generally in the deep tissues (spleen, liver, bone marrow), so a finger stick blood sample is no more likely to be positive than a venipuncture blood. There may be some differences with *P. falciparum*, but not sufficient to warrant eliminating venipunctures. The anticoagulant tube also provides additional blood for multiple thick and thin blood films and/or buffy coat films.

2. **What is the best anticoagulant to use for blood specimens?**
   Although heparin (green top) or EDTA (lavender top) can be used, EDTA is recommended as providing better organism morphology, particularly for *Plasmodium* spp. Blood collected using EDTA anticoagulant is acceptable; however, if the blood remains in the tube for >1-2 h, true stippling may not be visible within the infected RBCs (*Plasmodium vivax*, *Plasmodium ovale*). Also, when using anticoagulants, the proper ratio between blood and anticoagulant is necessary for good organism morphology. The lavender top tube should be filled with blood to provide the proper blood/anticoagulant ratio. Heparin can also be used, but EDTA is preferred. Finger stick blood is recommended when the volume of blood required is minimal (no other hematologic procedures have been ordered). The blood should be free-flowing when taken for smear preparation and should not be contaminated with alcohol used to clean the finger prior to the stick. However, finger stick blood is no longer commonly used in many parts of the world. When blood is collected in EDTA, specimens should be processed immediately after blood collection. **Parasite numbers may decrease if processing is delayed, even 4 to 6 hours.** Adhesion of the blood to the slide can be a problem if the ratio of anticoagulant to blood is high, the patient is anemic, or the blood was held in EDTA too long.

Organism	Morphology (Normal)	Morphology (Edta) Contact With Edta For >1-2 Hours
Plasmodium falciparum Rings Gametocytes	Typical, small to medium rings; double rings/cell Crescent-shaped	Numbers tend to remain constant; all parasites continue to grow in EDTA Gametocytes may round up and be confused with other species
Plasmodium vivax Trophozoites Schüffner's Dots	Ameboid trophozoites, for both early and late rings Typical dots appear in late rings; present throughout the rest of the life cycle stages	May round up and lose their characteristic shape. Stippling (Schüffner's dots) may not be visible at all, including all life cycle stages (regardless of buffer pH used). **NOTE**: If slides are prepared soon after collection in EDTA, the dots will be seen after staining; however, if slides are prepared after the blood has been in contact with EDTA for several hours, the dots may not be visible after staining.
Plasmodium spp. Male gametocytes	No exflagellation	As blood cools and becomes oxygenated, the parasites "think" they are now in the mosquito (this cycle may continue). The male gametocyte may exflagellate; microgametes may resemble *Borrelia* (also related to pH and pCO_2 – lid left off tube)

3. **Why do new slides have to be precleaned with alcohol prior to use?**
   Even new slides will be coated with a very fine layer of oil (to allow the slides to be "pulled apart" one from the other) - by removing the coating, the blood will flow more smoothly over the glass during blood film preparation. "Holes" in the blood film are evidence of oil or grease on the slide. **Use Standard Precautions**: remember both slides and spreaders must be held on the edges and not on any part of the slide that will come in contact with blood.

4. **When should blood specimens be drawn for a suspect malaria diagnosis?**
   The majority of patients we see in the US with malaria have never been exposed to the organism before; therefore they have no antibody and when they present **they do NOT have a synchronized fever cycle**. These immunologically naïve patients may present with nonspecific symptoms that can mimic many other diseases. The rule of thumb is to **draw immediately**; do not wait for some "magic" periodic cycle that may never appear. Patients with a very low parasitemia with *P. falciparum* can become quite ill before they have any type of fever cycle or gametocytes. With any patient where malaria is suspect or the patient has a FUO (fever of unknown origin), blood should be drawn and both thick and thin blood films prepared and examined immediately. **This request is always considered a STAT request.** In endemic areas of the world where people have been exposed to the parasite before (have some antibody), they may not become symptomatic until they actually have some sort of periodic fever cycle. However, you should always use the general guideline to "draw" immediately. Also, remember that one set of negative blood films does not rule out malaria. If the first set (both thick and thin films) is negative, you can recommend that additional blood films be drawn in about 4-6 hours. Also, any decision to delay treatment should be left to the physician, not the laboratory.

Specimen Processing

1. **Why is it important that the EDTA blood be processed as quickly as possible?**
   If a tube of blood containing EDTA cools to room temperature and the cap has been removed, several parasite changes can occur. The parasites within the RBCs will respond as if they were now in the mosquito after being taken in with a blood meal. The morphology of these changes in the life cycle and within the RBCs can cause confusion when examining blood films prepared from this blood.
   A. Stippling (Schüffner's dots) may not be visible.
   B. The male gametocyte (if present) may exflagellate.
   C. The ookinetes of *Plasmodium* species other than *P. falciparum* may develop as if they were in the mosquito and may mimic the crescent-shaped gametocytes of *P. falciparum*.
   D. Smears left longer than 24 h can autofix; if this occurs, lysis of the RBCs will be difficult, if not impossible, to achieve. This tends to occur more quickly in warm, humid climates.
   E. Thick smears can be dried in a 37ºC incubator for 10-15 min without fixation of the RBCs; **DO NOT GO BEYOND 15 MIN USING THIS METHOD.**

2. **Why is it important to keep thick films from getting hot (heat fixation)?**
   Heat will fix the RBCs and they subsequently will not lyse in the staining process. (See 1, E above).

3. **What should you do if you have blood films that can't be stained for several days?**
   If staining with Giemsa will be delayed for more than 3 days or if the film will be stained with Wright's stain, lyse the RBCs in the thick film by placing the slide in buffered water (pH 7.0 to 7.2) for 10 min, remove it from the water, and place it in a vertical position to air dry. The laked thick films and thin films should be dipped in absolute methanol and placed in a vertical position to air dry. This is particularly important if the blood films will be stored for days or weeks prior to staining.

Diagnostic Methods

1. **Why is it important to always examine both thick and thin blood films prior to reporting the specimen as negative for blood parasites?**
   Thick blood films allow a larger amount of blood to be examined, which increases the possibility of detecting light infections. However, only experienced workers can usually make species identification by thick film, particularly for malaria parasites. The morphologic characteristics of blood parasites are best seen in thin films.

2. **What are the advantages of the thick blood film?**
   The aims of the preparation of a thick film is to have a drop blood with 20 or 30 layers of red bloods on the slide, then to lyse the red cells, wash off the hemoglobin and stain the parasites, which remain intact in the process. As a consequence, red blood cells will not normally be visible, but white blood cells and parasites will be. It is essential to get used to the characteristics of malaria parasites and know what to look for on such a preparation. An experienced microscopist should be able to detect 20 parasites per microliter of blood (i.e. a parasitemia of 0.0001%) having examined 100 fields in 10 minutes. A greater volume of blood can be examined in the same amount of time taken for examination of the thin blood film. The presence of phagocytized malaria pigment within WBCs (particularly in cases with low levels of parasitemia) can be very helpful. Occasionally, one can see Schüffner's dots in a thick film.

3. **What are the disadvantages of the thick blood film?**
   Due to lysis of the RBCs in processing thick blood films, the thick film cannot be used to compare the size of the parasite within the RBC or to compare the size of the infected RBC to the uninfected RBCs. Recognition of organism distortion and ID to species are generally more difficult from the thick film.

4. **What are some of the problems associated with thick blood films?**
   The thick film flakes off during the staining process.
   a. Film was not dry.
   b. Film did not dry evenly.
   c. Blood film was too thick (refer to combination thick/thin blood film protocol).
   d. The blood was too diluted with anticoagulant or the patient was anemic (centrifuge blood at 500 x g for 1 min and repeat the thick film).
   e. Slides were greasy or dirty.
   f. Blood was in EDTA too long (prepare thick films thinner than normal; dry for a longer period of time).
   The thick film does not stain adequately.
   g. Blood film was too thick.
   h. Staining solution was too dilute or staining time too short.

5. What are some of the tips for improving thick blood films?
After the thick films are dry, fix the smear with acetone for a few seconds (**ONLY dip twice**), then air dry the film. This approach will improve the durability of the thick film, and will **NOT** interfere with the lysis of the RBCs. These films tend to have a clean background, making the parasites easier to see. Overall adherence to the slide is enhanced using this approach.

6. How do you prepare thin blood films?
Keep the spreader at about a 40-45º angle to allow blood to flow to ALMOST THE EDGE OF THE SLIDE. Lower the angle to about 30-35º to pull the film in one smooth motion (Figure 1). The finished blood film should appear as a feathered edge in the center of the slide, having a FREE MARGIN on either side. Before fixing the thin blood films in absolute methanol, the film must be completely dry. If slides must be stored unfixed, they should be frozen. Fixation too long (>30-45 sec): stippling may be reduced. Fixation too short: RBCs may be distorted (crescent-shaped) or RBCs may be partially lysed. FIXATION: It is better to use dispensing bottle for methanol, rather than a Coplin jar, in which the methanol will pick up water from the air. Methanol used on one day (Coplin jar) should not be reused the next day – begin with fresh stock.

7. What are the advantages of the thin blood film? .
The RBC morphology can be seen, as well as the size relationship of the parasite within the infected RBC. The sizes of the infected RBCs can be compared to that of the uninfected RBCs. It is much easier to identify malaria organisms to the species level using the thin blood film. The parasitemia can be calculated much easier from the thin blood film than the thick film.

8. What are the disadvantages of the thin blood film?
This method has a much lower sensitivity than the thick blood film; thus, infections with a low parasitemia may be missed.

9. What are some of the problems associated with thin blood films? Poorly prepared thick and thin blood films can be seen in Figure 2.

Films are too thick.

a. Too large a drop of blood was used.
b. The RBCs may be crowded together with no thin feathered edge.
c. The film was prepared too quickly or with too large an angle >30-35º.

Films are too thin.

d. Blood from anemic patients will not spread well; spread the blood more quickly. Allow the blood to settle or centrifuge, remove some plasma, repeat the thin film.
e. Angle between the spreader and slide was too small <30º.

Smears have ragged edges.

f. The drop of blood may have been in front of the spreader.
g. The slides were greasy or dirty.
h. The spreader slide was chipped.
i. The spreader slide was reused (use a new slide each time).
j. If the blood contained no anticoagulant, delay in spreading may have led to fibrin formation, causing streaking.
k. May also be due to high plasma fibrinogen level and NOT TECHNIQUE.

10. How does one prepare a combination thick/thin blood film?
The combination thick-thin blood film provides both options on one glass slide and the slide can be stained as either a thick or thin blood film (Figure 3). If fixed prior to staining, then the smear will be read as a thin blood film; if RBCs are lysed during staining, the preparation will be read as a thick blood film (parasites, platelets, WBCs). This combination blood film dries more rapidly than the traditional thick blood film, thus allowing staining and examination to proceed with very little waiting time for the slide(s) to dry.

A. Place a drop (30 to 40 µl) of blood onto one end of a clean slide about 0.5 in. from the end.
B. Using an applicator stick lying across the glass slide and keeping the applicator in contact with the blood and glass, rotate (do not "roll") the stick in a circular motion while moving the stick down the glass slide to the opposite end.
C. The appearance of the blood smear should be alternate thick and thin areas of blood.
D. Immediately place the film over some small print and be sure that the print is just barely readable.
E. Allow the film to air dry horizontally and protected from dust for at least 30 min to 1 h. Do not attempt to speed the drying process by applying any type of heat, because the heat will fix the RBCs and they subsequently will not lyse in the staining process.
F. This slide can be stained as either a thick or thin blood film.

11. How should malaria smears be stained with Giemsa Stain?
Giemsa is a mixture of eosin and methylene blue. Stock solutions of Giemsa may be purchased commercially. Some brands are better than others. The stock solution of Giemsa stain is easily prepared from commercially available Giemsa powder.

• Dilutions of Giemsa stain
Stock solutions of Giemsa stain must always be diluted by mixing an appropriate amount of stain with distilled neutral or slightly alkaline water; buffered saline is preferred because it provides a cleaner background and better preservation of parasite morphology. Although most people do not filter the working stain prior to use (if using a Coplin jar), results are better overall if the working stain is filtered through Whatman no.1 filter paper immediately before use. Make sure to use absolute methanol (acetone-free) for fixing thin blood films. Stock buffered water is stable for 1 year at room temperature. Working stock buffered water is stable for 1 month at room temperature. Stock Giemsa stain is stable at room temperature indefinitely; stock stain appears to improve with age (similar to that seen with iron-hematoxylin stains). A 45-60 minute staining time appears to work better than 15 min; staining times will depend on stain dilution. Some workers feel the use of a 10% Triton solution is helpful, while some feel it is detrimental to the overall quality.

12. How should you handle a delay between thick film preparation and staining?
Thick films can be preserved, particularly if there will be a delay prior to staining.
Dip the slides in a buffered methylene blue solution (0.65% for 1-2 seconds and allow the slides to dry.

a. Methylene blue 0.65 g
Di-sodium hydrogen phosphate Na_2HPO_4 2.0 g
Potassium di-hydrogen phosphate KH_2PO_4 0.65 g
Distilled water 1.0 L
Mix and store in a brown-stoppered bottle.

The benefits of such an approach are as follows:
b. The RBCs are hemolyzed
c. Color is introduced into the cytoplasm of the parasites
d. Prestaining with methylene blue helps preserve the organisms on the smear

13. Can blood stains other than Giemsa stain be used to stain the blood films?

The detection of parasites in the blood has been made possible by the use of Romanovsky-type differential stains which selectively color the nuclear material red and the cytoplasm blue. This reaction takes place under optimal pH conditions: rather than using a neutral pH, optically perfect results are obtained using the slightly basic pH of 7.2 (due to the optical dominance of red pigmentation). Although for many years, Giemsa stain has been the stain of choice, the parasites can also be seen on blood films stained with Wright's stain, a Wright/Giemsa combination stain or one of the more rapid stains such as Diff-Quik (American Scientific Products, McGraw Park, IL), Wright's Dip Stat Stain (Medical Chemical Corp., Torrance, CA), or Field's stain. **It is more appropriate to use a stain with which you are familiar, rather than Giemsa which is somewhat more complicated to use. PMNs will serve as the QC organism for any of the blood stains. Any parasites present will stain like the PMNs, regardless of the stain used**. Also, the CAP checklist does not mandate the use of Giemsa stain.

14. How should malaria blood films (both thick and thin films) be examined?

A minimum of 300 oil immersion fields using the 100X objective should be examined. The blood film can be scanned using a 50X or 60X oil immersion lens, but final reporting of the results should be based on the use of the 100X oil immersion lens for a total magnification of X1,000.

15. What type of QC slides should be used for blood parasite work?

Regardless of the stain you are using (Giemsa, Wright, Wright/Giemsa, rapid stains), **your QC slide is the actual slide you are staining**. This approach to QC is acceptable to CAP, as well. Any parasites present will stain like WBCs, so your QC is built into the system. A good source for teaching slides is: Meridian Bioscience, Inc. They have slides and specimens available for sale. Contact them for a brochure: (800) 543-1980 Ext 335.

Organism Identification

1. Why is it so important to "rule out" infections with *P. falciparum*?

P. falciparum causes more serious disease than the other three species (*P. vivax, P. ovale, and P. malariae*). *P. falciparum* tends to invade all ages of RBCs, and the proportion of infected cells may exceed 50%. **ALSO REMEMBER THAT INFECTIONS WITH *PLASMODIUM KNOWLESI* ARE CONSIDERED AS SERIOUS AS THOSE WITH *P. FALCIPARUM*.** Schizogony occurs in the internal organs (spleen, liver, bone marrow, etc.) rather than in the circulating blood. Ischemia caused by the plugging of vessels within these organs by masses of parasitized RBCs will produce various symptoms, depending on the organ involved.

Onset of a *P. falciparum* malaria attack occurs from 8 to 12 days after infection and is preceded by 3 to 4 days of vague symptoms such as aches, pains, headache, fatigue, anorexia, or nausea. The onset is characterized by fever, a more severe headache, and nausea and vomiting, with occasional severe epigastric pain. There may be only a feeling of chilliness at the onset of fever. Periodicity of the cycle will not be established during the early stages, and the presumptive diagnosis may be totally unrelated to a possible malaria infection. If the fever does develop a synchronous cycle, it is usually a cycle of somewhat less than 48 h. An untreated primary attack of *P. falciparum* malaria usually ends within 2 to 3 weeks. True relapses from the liver do not occur, and after a year, recrudescences are rare. *Severe or fatal complications of* P. falciparum *malaria can occur at any time during the infection and are related to the plugging of vessels in the internal organs, the symptoms depending on the organ(s) involved.* **NOTE: The primary objective when performing blood film examination for parasites is to "rule out *P. falciparum*."**

2. What are some of the problems associated with the differentiation between *Plasmodium* spp. and *Babesia* spp.? Can *Babesia* spp. cause serious infections?

The ring forms of all four species of *Plasmodium* can mimic the ring forms of *Babesia* spp. Multiple rings per cell are more typical of *P. falciparum* than of the other four species causing human malaria. *Babesia* rings are often numerous, of smaller sizes, and tend to be very pleomorphic, while those of *P. falciparum* tend to be fewer in number and more consistent in size and shape. Remember that P. falciparum rings may appear somewhat larger if the blood has been drawn in EDTA and there is any lag time prior to thick and thin film preparation. Differentiation between *Plasmodium* spp. and *Babesia* spp. may be impossible without examining a thin blood film (rather than a thick blood film). Often, the parasitemia in a *Babesia* infection may be heavier than that seen with *P. falciparum*, particularly when a patient presents early in the infection. It is also important to remember that some of the *Babesia* spp. can cause severe illness [*B. divergens* from Europe, etc. (42% mortality), *Babesia* from California, Oregon, Washington, Missouri], while some infections such as those caused by *B. microti* cause less serious or subclinical infections. **HOWEVER,** ANY of the *Babesia* spp. can cause severe disease in immunocompromised patients, particularly those patients who have undergone splenectomy.

3. What is the significance of finding only ring forms on two sets of blood films drawn 6 h apart?

Remember that all of the life cycle stages (rings, developing trophozoites, early schizonts, late schizonts, mature schizonts, and gametocytes) can be seen on the blood films in infections with *P. vivax, P. ovale,* and *P. malariae*. Due to unique characteristics of the life cycle, only rings and gametocytes (and occasional mature schizonts) are seen in the peripheral blood with a *P. falciparum* infection. **Therefore, if you see two sets of blood films (collected 6 h apart) that contain ring forms only, there is an excellent chance the patient is infected with *P. falciparum*, the most serious of the four *Plasmodium* spp. infections**.

4. Why aren't gametocytes of *P. falciparum* seen in many patients presenting to the Emergency Room (ER)?

Many patients present to the ER early in the infection prior to the formation of the gametocytes; thus the diagnosis must be made based on seeing the ring forms only. As you can imagine, identification of *Plasmodium* to the species level can be very difficult when rings only are present. These patients tend to be travelers who have had no prior exposure to *P. falciparum* (immunologically naïve) and who become symptomatic very early after being infected. It normally takes approximately 10 days for the crescent-shaped *P. falciparum* gametocytes to form.

Reporting

1. **Why is it important to identify malaria organisms to the species level?**
   Since *P. falciparum* and *P. knowlesi* can cause severe disease and death, it is very important for the physician to know whether this infection can be "ruled out." It is also important to know if any of the other three species are present, particularly *P. vivax* or *P. ovale*, which would require therapy for both the liver and RBC stages due to potential relapse from the liver stages. It is also important because of potential drug resistance [chloroquine, *P. falciparum, P. vivax*; primaquine tolerance/resistance, *P. vivax* (rare, but documented)].

2. **How should a positive malaria blood film be reported?**
   A. Using the thin blood film method, report the percentage of parasite-infected RBCs per 100 RBCs counted.
   Example: *Plasmodium falciparum*, parasitemia = 0.01%
   B. Using the thick/thin blood film method, report the number of parasites per µl of blood.
   C. Example: *Plasmodium falciparum*, parasitemia = 10,000 per µl of blood

3. **How should results be reported if *Plasmodium* spp. parasites are seen, but *P. falciparum* infection cannot be ruled out?**
   It is important to convey to the physician that *P. falciparum* cannot be rule out; therapy may be initiated on the assumption that this species might be present. The report should read:
   ***Plasmodium* spp. present; unable to "rule out" *Plasmodium falciparum*.**

4. **How often do mixed infections occur and how should they be reported?**
   It is important to remember that mixed infections are much more common than suspected and/or reported. When rings are present, along with other developing stages (*P. vivax, P. ovale, P. malariae*) always look for the presence of two populations of ring forms, one of which might be *P. falciparum*! The report should read:
   ***Plasmodium* spp. present, possible mixed infection; unable to "rule out" *P. falciparum*.**
   Another report example might be:
   ***Plasmodium vivax* rings, developing schizonts, and gametocytes; possible mixed infection: unable to "rule out" *P. falciparum*.**

5. **Why is it important to report the *Plasmodium* spp. stages seen in the blood films?** Often patients who have been diagnosed with *Plasmodium* spp. who are not suspected of having drug resistant malaria (*P. falciparum, P. vivax*) are treated with chloroquine. Chloroquine will not eliminate any gametocytes present, and there are mosquitoes within the United States that can transmit malaria if they take a blood meal from an individual with gametocytes in the blood. Thus, it is important for the physician to know which stages are present in the blood (rings, developing trophs, schizonts, and/or gametocytes).

6. **How do parasitemia and malaria severity correlate?**
   The following percentages are helpful in interpretation of malaria severity:

   - Parasitemia of >10,000/µl = heavy infection
   - 0.002%, 100/µl, symptomatic below this level
   - 0.2%, 10,000/µl, immune patients symptomatic
   - 0.1%, 5,000/µl, sensitivity of rapid malaria test (BinaxNOW) at close to 100%; will decrease significantly if parasitemia is below 0.1%
   - 2%. 100,000/µl, maximum parasitemia for *P. vivax, P. ovale* – rarely go above 2%
   - 2-5%, up to 250,000/µl, severe malaria, mortality
   - 10%, 500,000/µl, exchange transfusion

Proficiency Testing

1. **How should blood films be examined for proficiency testing (PT)?**
   Since you have no idea what organisms might be present, always review the blood films using the 10X objective (**entire** slide). This examination is likely to reveal any microfilariae that are present; however, small parasites like *Plasmodium* and *Babesia* may be missed. Before reporting the smear as negative, examine at least 300 oil immersion fields using the 100X oil immersion lens.

2. **Do Proficiency Testing (PT) blood films match those seen from actual patients?**
   Yes and no! Blood films for PT are actual patient specimens, however, they may have a higher parasitemia than is seen in many patients reporting to the ER, clinic, etc. Often, smears contain a higher parasitemia than is commonly seen. So, the PT smears represent a mix, some of which are fairly typical and some of which have a high number of organisms present. **As in the case of a traveler, the thin blood film may appear to be negative, while the thick film will be positive!**

3. **What blood parasites might be seen in PT specimens?**
   The following parasites may be seen in PT specimens:
   Plasmodium falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi (less likely) mixed malaria infections, *Babesia* spp., *Trypanosoma cruzi*, *Trypanosoma rhodesiense/gambiense, Leishmania* spp., and *microfilariae.*

General Questions

1. **What stains are recommended for staining microfilariae?**
   Although Giemsa stain is generally recommended, it will not stain the sheath of *Wuchereria bancrofti*; hematoxylin-based stains (Delafield's) are recommended. Also, the sheath of *Brugia malayi* will stain pink with Giemsa stain.

2. **What other methods can be used for the identification of blood parasites?**
   Concentrations (trypanosomes, microfilariae, leishmaniae)
   Buffy coat thick and thin blood films (all blood parasites, including *Plasmodium* spp.)
   Skin biopsies or bone marrow aspirates (Giemsa or other blood stains) (leishmaniae)
   Rapid methods/dipstick formats (malaria, microfilariae) (Figure 4). **NOTE: The Inverness/Binax rapid test for *Plasmodium falciparum* and *Plasmodium* spp. is FDA approved (June, 2007); however, a positive external control is also available for customers.** The Binax rapid test is as sensitive as a good microscopist; however, often these skilled

individuals may not be available on off-hour shifts. However, it is important to recognize the pros and cons of the rapid test when compared to microscopic examination of blood films, particularly in travelers in whom the parasitemia may be considerably less than 0.1%.

3. **How good are serologies for malaria; when should they be performed?**
Malaria serologies are available through CDC. They always test with antigens of all 5 species, but the reactions are not species specific - there is a lot of cross-reactivity. *Plasmodium* spp. can be determined in about 80% of NON-IMMUNE patients in their PRIMARY infection (U.S. Army during Vietnam War), but cannot determine species in patients with long-term exposure and multiple infections (i.e., Africans). CDC does not recommend serology for identification to the species level; PCR is the best tool for this purpose if pre-treatment EDTA blood is available.

4. **How do you categorize malaria resistance (seen in both *P. falciparum* and *P. vivax*)?**

Resistance Definition	Comments
Sensitive	From initiation of therapy, asexual parasites are cleared by day 6; no evidence of recrudescence up to day 28 ► Peripheral blood films appear to go from positive to negative very quickly (can be a change from one draw to the second draw 6 h later).
Resistance type I (RI)	From initiation of therapy, asexual parasites have cleared for at least two consecutive days (the latest day being day 6); recrudescence follows ► Parasite count initially drops and blood films appear to be negative; patient should be monitored for a period of days, particularly if drug-resistant *P. falciparum* suspected
Resistance type II (RII)	Within 48h of initiation of therapy, marked reduction of asexual parasitemia to <25% of pretreatment count; however, no subsequent disappearance of parasitemia (smear positive on day 6) ► Patient appears to be improving; parasite count drops, but blood films always appear positive.
Resistance type III (RIII)	Modest reduction in parasitemia may be seen; no change or increase in parasitemia seen during first 48 h after treatment; no clearing of asexual parasites ► In some cases, the parasite count continues to increase with no visible decrease at any time; blood films show overall parasite increase.

5. **How soon after initiation of therapy for *P. vivax* malaria would one expect to see clearance in blood smears? How soon should follow-up blood smears be submitted for evaluation after therapy?**
Although the number of cases we see in the United States is certainly less than in endemic areas, we begin to see a drop in parasite numbers very quickly (within a few hours) after the initiation of therapy. If an individual presents in the ER, the parasitemia is often below 1-2% (0.1 – 0.01%); the patient is given therapy and is not admitted to the hospital (in the case of *P. vivax, P. malariae,* or *P. ovale*). If the organism is identified as *P. falciparum, P. knowlesi,* or a mix (most common would be *P. falciparum/P. vivax*), the patient will be admitted. In some cases, we do not receive blood for followup examination (from ER patients where *P. falciparum* and *P. knowlesi* have been ruled out). When treating *P. falciparum*, we receive blood samples approximately every 4-6 hours for routine checks (patients will be admitted to the hospital for treatment). Although the number of resistant *P. vivax* cases is small, it has been confirmed, so this is always a consideration, as are mixed infections with *P. falciparum* and *P. vivax*.

6. **Where can one get PCR performed for the malaria diagnosis and identification to the species level?**
CDC performs PCR for malaria, which identifies the organisms to the species level. CDC requires about ½ ml of pre-treatment EDTA blood. Also if possible they would like to review both thick and thin smears if extra blood films are available. Specimens can be sent overnight to:

Microbiologist
Division of Parasitic Diseases
Centers for Disease Control and Prevention
Building 109, Room 1302
4770 Buford Highway NE
Atlanta, GA 30341
Phone: 770-488-7044

7. **What is the current status of rapid testing for malaria?**
This issue has been a difficult one to resolve in the United States. Currently, the BINAX rapid test was approved by the FDA in June, 2007 (Figure 4) (see table below). An external positive control is also available for customers. Certainly the use of these rapid tests would be a tremendous advantage for personnel on the night shift. However, **if the test was negative, it would not rule out a *Plasmodium* spp. infection, and thick and thin blood films would need to be prepared and examined STAT.** If positive, then followup would need to be performed on slides to rule in/out mixed infections. All kits (published data) indicate that the kits are helpful, but they are no better and somewhat less accurate than a good microscopist. However, as we all know, these individuals are often not available on off-hour shifts. Many places have solved the problem by just bringing someone in to prepare and read slides on off hours. Often, the PM shifts prepare and stain smears (Wright-Giemsa or one of the rapid stains) and another individual would examine the thick and thin stained blood films. We tried to expose our residents/post-docs/etc. to these kits and found them to be helpful. **However, when dealing with immunologically naïve patients with very low parasitemia values, it would be easy to miss an infection unless careful examination of the thick films was performed. REMEMBER, THE RAPID TEST WILL NOT TAKE THE PLACE OF CAREFUL EXAMINATION OF BOTH THICK AND THIN BLOOD FILMS**.

% Parasitemia	Comments
Parasitemia of >10,000/µl = heavy infection	
0.0001-0.0004, 5-20/µl	Required for a positive thick blood film
0.002%, 100/µl	Immunologically naïve patients may be symptomatic below this level
0.02%, 1,000/µl	Can be seen in travelers in emergency room (symptomatic very early with low parasitemia = immunologically naïve)
0.1%, 5000/µl	**BinaxNOW rapid lateral flow method** = level of sensitivity below this percentage drops significantly – problem with travelers (0.01% or less)
0.2%, 10,000/µl	Immune patients are symptomatic
2%, 100,000/µl	Maximum parasitemia for *P. vivax* and *P. ovale* – rarely goes >2%
2-5%, up to 250,000/µl	Severe malaria, high mortality
10%, 500,000/µl	Exchange transfusion usually required

8. Can mosquitoes be infected with more than one species of malaria?

"There appears to be no barrier to infection of *Anopheles* with mixed *Plasmodium* species. Mosquitoes doubly infected with *P. falciparum* and *P. vivax* were able to transmit both species to humans following deliberate feeding on volunteers (Boyd et al, 1937). In natural situations, however, suppressive effects in the human host may lead to overlapping waves of gametocytes of different species, so that there is a tendency for mosquitoes to be infected with only one species. In some cases there may be specific suppression of sporogony of one species compared to the other. For example, in areas where *P. falciparum* and *P. malariae* are sympatric, the cold temperatures associated with altitude may be disadvantageous to *P. falciparum*, which requires a higher temperature for sporogony than *P. malariae*. This may lead to seasonal changes in prevalence of the two species (de Zuluerta et al, 1964).

9. What is the general status of PCR for leishmaniasis?

PCR for leishmaniasis has been through some very rigorous validation at the Leishmaniasis lab at Walter Reed Army Institute of Research and is excellent. Contact Dr. Glen Wortmann at glenn.wortmann@us.army.mil

10. Are serologies available for the African trypanosomes?

CDC doesn't do PCR for African trypanosomiasis. It is unnecessary for diagnosing *T. b. rhodesiense* (parasites easily detectable by microscopy). For *T. b. gambiense*, it would need to be arranged a laboratory in Europe. It is viewed as a research tool only. The CDC consultant is: Dr. Anne Moore (770 488-7776),

Suggested Reading

Garcia, L.S. (ed), 2010. *Clinical Microbiology Procedures Handbook*, 3rd ed. ASM Press, Washington, D.C., Parasitology Section in Vol 2 of 3 vols.

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Garcia, L.S. (Coordinating Editor), 2003. *Selection and Use of Laboratory Procedures for Diagnosis of Parasitic Infections of the Gastrointestinal Tract*, Cumitech 30A, ASM Press, Washington, D.C.

Hanson, K. L., and C. P. Cartwright. 2001. Use of an enzyme immunoassay does not eliminate the need to analyze multiple stool specimens for sensitive detection of Giardia lamblia. *J. Clin. Microbiol*. **39**:474-477.

Isenberg, H.D. (ed.), 2004. *Clinical Microbiology Procedures Handbook*, 2nd ed. ASM Press, Washington, D.C., Parasitology Section in Vol 2 of 3 vols.

National Committee for Clinical Laboratory Standards, 2000, *Use of blood film examination for parasites*. Approved Guideline M15-A, National Committee for Clinical Laboratory Standards, Villanova, PA,

National Committee for Clinical Laboratory Standards, 1997, *Procedures for the recovery and identification of parasites from the intestinal tract*, 2nd Ed., Approved Guideline, M28-A National Committee for Clinical Laboratory Standards, Villanova, PA.

Figure Legends:

Figure 1. The traditional method for preparing a thin blood film; the blood can be either "pushed" or "pulled" by the spreader slide (Illustration by Sharon Belkin.)

Figure 2. Poorly prepared thin and thick blood films (dirty slides, oil on slides, too thick preparations, poor spreading of the blood); organism morphology will be very poor on the stained films.

Figure 3. Method of thick-thin combination blood film preparation. (a) Position of drop of EDTA-containing blood. (b) Position of the applicator stick in contact with blood and glass slide. (c) Rotation of the applicator stick. (d) Completed thick-thin combination blood film prior to staining. (Illustration by Sharon Belkin.)

Figure 4. Rapid malaria test; top, the negative test shows the control line only; middle, control line plus the *Plasmodium vivax* line indicates the presence of a panspecific antigen (common to all *Plasmodium* spp., but most sensitive for *P. vivax* rather than *P. ovale* and *P. malariae*); bottom, control line, panspecific antigen line, and line specific for *Plasmodium falciparum* antigen presence .

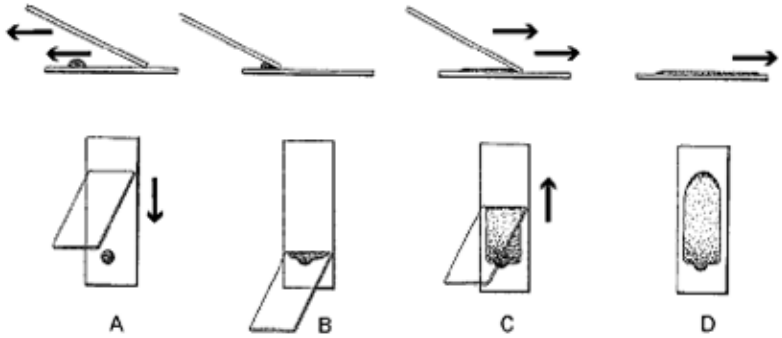


Figure 1

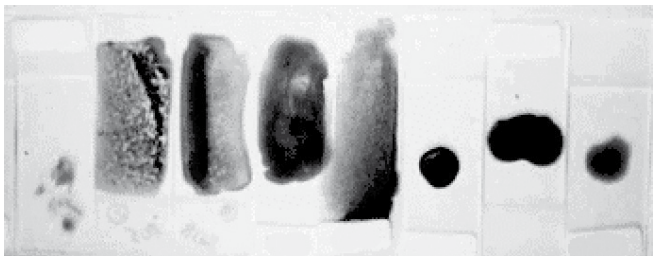


Figure 2

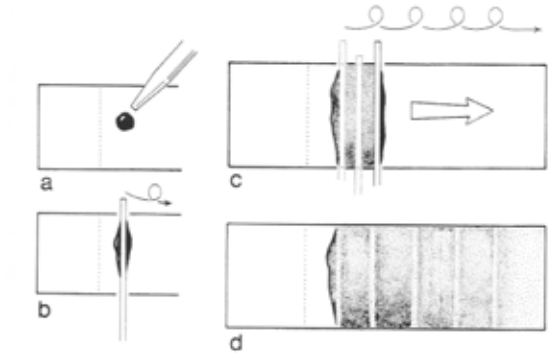


Figure 3

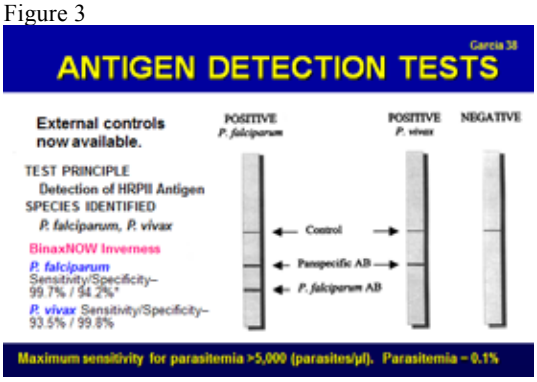
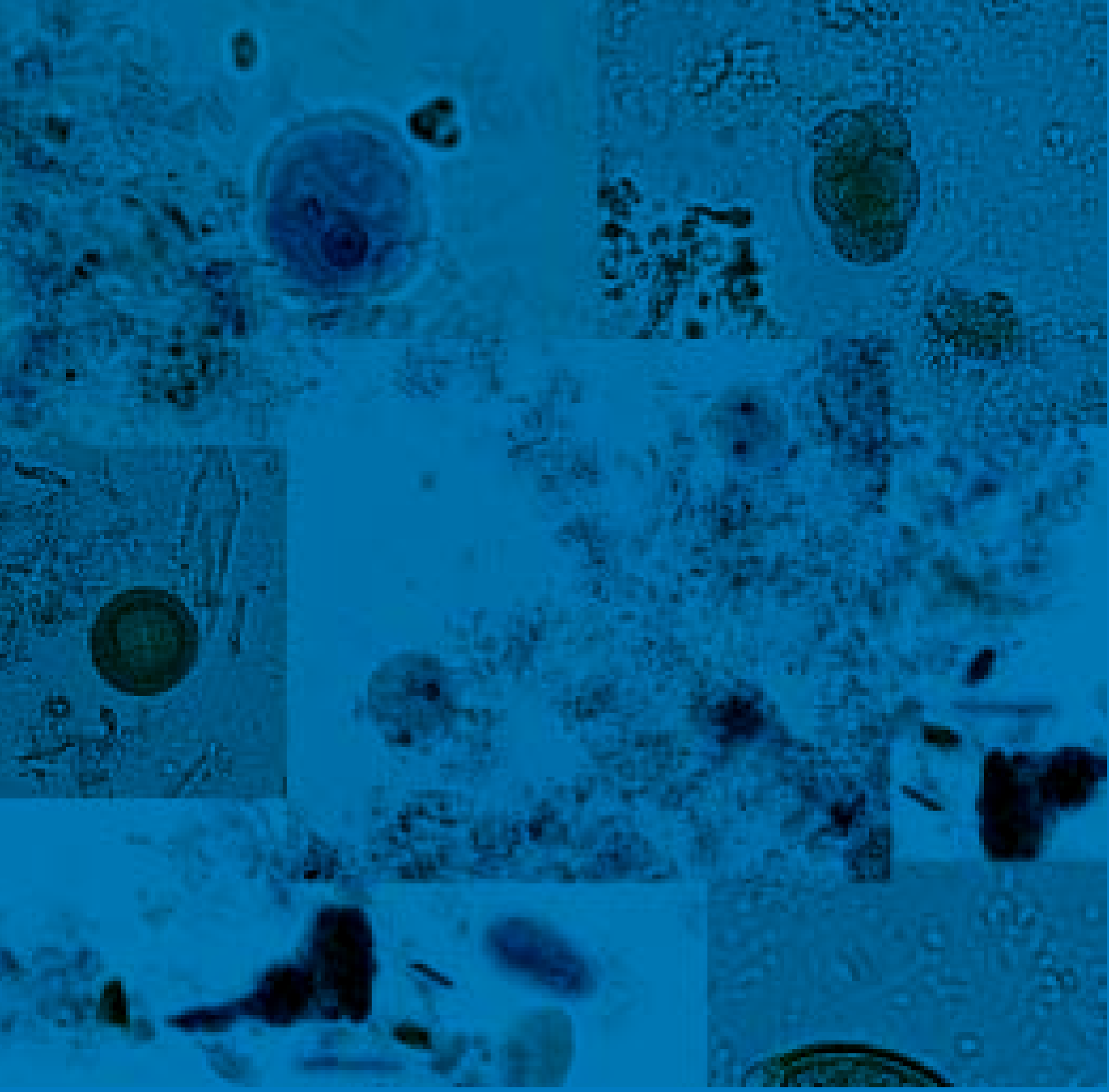


Figure 4 **ALSO SEE TABLE ON PAGE 17,18**

SENSITIVITY FOR ***Plasmodium falciparum*** **5000 = 0.1%**	**SENSITIVITY FOR** ***Plasmodium vivax*** **5000 = 0.1%**
Parasitemia Level % Sensitivity 95%CI	**Parasitemia Level % Sensitivity 95%CI**
> 5000 99.7% (326 / 327) 98 - 100%	> 5000 93.5% (462 / 494) 91 - 96%
1000 – 5000 99.2% (126 / 127) 96 - 100%	1000 – 5000 81.0% (277 / 342) 76 - 85%
500 – 1000 92.6% (25 / 27) 76 - 99%	500 – 1000 47.4% (37 / 78) 36 - 59%
100 – 500 89.2% (33 / 37) 75 - 97%	100 – 500 23.6% (34 / 144) 17 – 31%
0 – 100 53.9% (21 / 39) 37 - 70%	0 – 100 6.2% (8 / 129) 3 – 12%
Overall 95.3% (531 / 557) 93 - 97%	Overall 68.9% (818 / 1187) 66 - 72%



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