PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Ova & Parasite Exam Procedure

Department of Microbiology

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1.0 Clinical Significance

An ova and parasite (O&P) exam is a microscopic evaluation that is commonly used to look for parasites that have infected the lower digestive tract. Other specimen types may also be submitted to look for specific parasites.

2.0 Principle

When present in small numbers, parasites may be missed in a direct wet mount. A concentration procedure should be employed on all stools, whether the direct smear is positive or not. Because the preserved organisms will no longer exhibit motility, the direct wet smear is no longer considered a mandatory part of the routine ova and parasite examination. However, if fresh liquid specimens are delivered to the laboratory immediately, the direct wet smear, particularly on liquid stools should be performed.

Fecal specimens are concentrated using the Parasep® SF (solvent free) device. Specimens received in fixative can be transferred to the Parasep® device. Fresh specimens received from in house can be processed using Parasep® devices that are prefilled with fixative. During centrifugation, the Parasep® concentrator removes fat and debris from fecal specimens while efficiently collecting ova and parasites in the sediment. No formalin or ethyl acetate is used in this protocol and the sediment collected after a single centrifugation can be used to prepare the permanent smear and the concentrated wet prep.

The Gomori-Trichrome stain is used for making permanent stains. It provides 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.

3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiarized and trained to process samples and perform microscopic examination. Testing includes but is not limited to: macroscopic and microscopic specimen examination, QC checks, technical proficiency testing and competency assessment.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines. The reagents and/or chemicals that are used in this procedure may be hazardous to your health if handled incorrectly. More extensive information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Material Safety Data Sheet (MSDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Enteric pathogens
- Bloodborne pathogens
- Hazardous reagents

To perform this procedure, you must use:

- Gloves – must be worn when handling specimens and reagents.
- Laboratory Coat – must be worn when handling specimens and reagents.

Disinfectant following procedure:

- Bleach dilution sprayers or wipes can be used for on demand disinfectant.

Reference for spill/decontamination:

- MSDS
- Chemical hygiene plan

5.0 Specimen Information

1. Duodenal contents or sigmoidoscopy material.
2. Sputum, bronchoalveolar lavage, transtracheal aspirates for *Ascaris lumbricoides* larvae, *Strongyloides filariform* larvae, hookworm larvae, *Paragonimus westermani* ova, and *Echinococcus granulosus* hooklets, *Entamoeba histolytica* and *Cryptosporidium* spp.
3. Lung or liver aspirates for *Entamoeba histolytica* or *Echinococcus*.
4. Urine for *Schistosoma* spp. Peak egg excretion occurs between noon and 3 PM. Samples collected during this time or during a 24-h urine collection without preservatives, may be used for examination.
5. Freshly collected stool (for PSHMC inpatients only)
   - Pass the stool into any clean, dry container
   - Select a walnut sized portion of the stool from areas that are watery or bloody and place it into the clean, leak-proof container.
   - Label the container with the patient name, physician, and date and time of collection.
   - Transport the specimen to the laboratory immediately, (within 30 min) if stool is watery/liquid of collection.
   - A total of 3 specimens should be collected and submitted on separate days or collected and submitted every other day.
6. Preservative
   - If there is an expected delay in transport to the laboratory beyond 30 min, a stool preservative should be used.
   - Pass the stool into a clean, dry container.
   - Using the spoon attached to the fixative cap, collect small amounts of stool from areas that are mucoid, watery, or bloody, and place them into the vial. Use only one vial of preservative per specimen collection. Each vial must be filled with enough specimen so that the liquid reaches the Fill Line located on the label.
   - Tighten the cap securely to avoid leakage.
   - Shake the sample thoroughly to mix the specimen and preservative.
   - Label each container with the patient name, physician, and date and time of collection.
   - Place the vial(s) back into the bag, and seal.
   - Three specimens should be collected, collecting 24 h apart or every other day.

6.0 Materials

6.1 Preservatives Acceptable for Specimen Submission

- Unifix™
- Total-Fix™

6.2 Equipment

- Centrifuge
- Coplin jars labeled with contents and procedural time
- Light microscope with 10, 40, and 100 X objectives and a calibrated ocular micrometer

6.3 Consumables

- Parasep® concentrator devices without reagent (for preserved fecal specimens) or Parasep® devices prefilled with preservative and Triton X-100 (for fresh specimens)
- Transfer pipettes with a wide bore (Samco part no. 993)
- Paper towels
- Glass slide
- Coverslips

6.4 Reagents

- 0.85% Saline
- 5% Solution of Triton X-100 Surfact-Amps® Detergent

Prepare by making a 1:2 dilution of Triton X-100 (Thermo Scientific prod. # 85111) with saline or deionized water. Store at room temperature for up to one year.

- Citrisolv
- D'Antoni's Iodine
- Gomori-trichrome Stain Solution
- Microscope immersion oil

7.0 Interfering Substances

1. Fecal specimens should be collected prior to the administration of antibiotics or antidiarrheal agents.
2. Mineral oil, bismuth, and barium may interfere with the detection or identification of intestinal parasites.

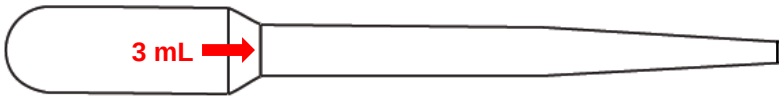
8.0 Procedure for Fecal Specimens

8.1 Direct Mount of Fresh, Unpreserved Stool

1. Examination must be carried out within 30 min of passage for liquid stools.
2. Place 1 drop of 0.85% saline on a glass slide. Add 1 drop of stool, mix, and cover slip. Examine the entire coverslip for motile trophozoites, larvae, and eggs.

8.2 Concentration of Preserved Fecal Specimens

1. Add 8 drops of 5% Triton X-100 to the preserved specimen.
2. Reseal the cap on the vial tightly. Vortex or shake the vial to emulsify the sample.
3. Transfer 3 mL of the emulsified stool into the mixing chamber. When using a Samco wide-bore transfer pipette, 3 mL can be measured by drawing specimen up to the base of the bulb.



4. Seal the Parasep® by screwing in the filter/sedimentation cone unit.
5. Label the top cone unit with an accession label.
6. Invert the Parasep® and centrifuge at 400 x *g* for 2 min.
7. Unscrew and discard the filter and mixing tube.
8. Pour off all the liquid above the sediment.
9. From the resulting sediment, prepare smear for permanent stain and wet mount.

8.3 Concentration of Fresh Fecal Specimens

1. Use the green scoop assembly to transfer one, slightly heaped spoonful of stool to a Parasep® pre-filled with 3.3 mL of preservative and Triton X-100.
2. Seal the Parasep® by screwing in the filter/sedimentation cone unit.
3. Vortex or shake to emulsify the sample with the sedimentation cone pointing upwards.
4. Label the top cone unit with an accession label.
5. Invert the Parasep® and centrifuge at 400 x *g* for 2 min.
6. Unscrew and discard the filter and mixing tube.
7. Pour off all the liquid above the sediment.
8. From the resulting sediment, prepare smear for permanent stain and wet mount.
9. Cap the tube to prevent drying.

8.4 Permanent Smears of Stool Specimens

8.4.1 Smear Preparation for Permanent Smears

1. Re-suspend the sediment.
2. Prepare a slide for permanent staining by adding a small sample of the suspended sediment to the slide. The sediment can also be used to prepare smears for modified acid-fast staining for coccidia.
3. Spread the sample over the slide to prepare a thin smear which varies in thickness. Allow to dry for a minimum of 30 min (60 min if slide is thicker) in a 37°C incubator or slide warmer. Smear will appear opaque when dry. Do not use a heating block. The higher temperature will be detrimental to any organisms present.

8.4.2 Staining Permanent Smears

1. Place slide in Trichrome stain jar for 5 min.
2. Rinse in 95% ethyl alcohol jar for 10 s.
3. Place in 95% ethyl alcohol jar for 4 min.
4. Place in Citrisolv/95% Ethyl alcohol jar for 4 min.
5. Place in Citrisolv jar for 5 min.
6. Remove Slide, drain excess, and allow to air dry.

8.4.3 Examination of Permanent Smears

1. The trichrome stain should be read under oil immersion. Examine for presence of parasite egg, ova, or larvae after reviewing 300 oil/fields.
2. The use of a calibrated ocular objective will aid in definitive identification.
3. The cytoplasm of cysts and trophozoites is blue-green, tinged with purple. Occasionally, *E. coli* cysts may stain slightly more purplish than cyst of other species. The nuclear chromatin, chromatoid bodies, and ingested red cells, and bacteria stain red or purplish red. Other ingested particles, such as yeast or molds, generally stain green, but variations frequently occur in the color reaction of ingested particles. Background usually stains red and contrasts strongly with the green background. The cytoplasm of pus and tissue cells, however, does stain more greenish than that of the protozoa.
4. Thick and thin areas should be observed. Organisms are more easily located in the thin areas, but in some preparations these portions may be poorly stained or the organisms poorly differentiated. In these cases, the thicker portions would be of greater diagnostic value.

8.5 Wet Mount of Concentrated Stool

8.5.1 Preparation & Examination of Concentrated Wet Mount

1. If the sediment is still somewhat solid, add a drop or two of saline to the sediment, mix, and add a small amount of material to a slide. Mix with 1 drop of D'Antoni's Iodine, add a coverslip, and examine under reduced light.

2. Systematically scan entire coverslip area with the low 10x objective looking for eggs and larvae. If something suspicious is seen, the 40x objective can be used to study detail. At least one-third of the coverslip should be examined under high dry power even if nothing suspicious is seen.
3. Consult appropriate reference books or the CDC DPDx website for definitive identification. Refer to supervisor or director if questionable identification.

8.6 Duodenal Contents or Sigmoidoscopy Material

Duodenal contents or sigmoidoscopy material must be examined by direct wet mount method when received within 30 min. Specifically look for motile *Giardia lamblia* trophozoites while searching for parasites. A concentration procedure can be performed if quantity is sufficient. A permanent trichrome stain must be prepared and reported. Usually, parasites such as *Giardia* will stick to the mucous portions. If mucous is present, it should be included in the direct mount examination and permanent trichrome stain.

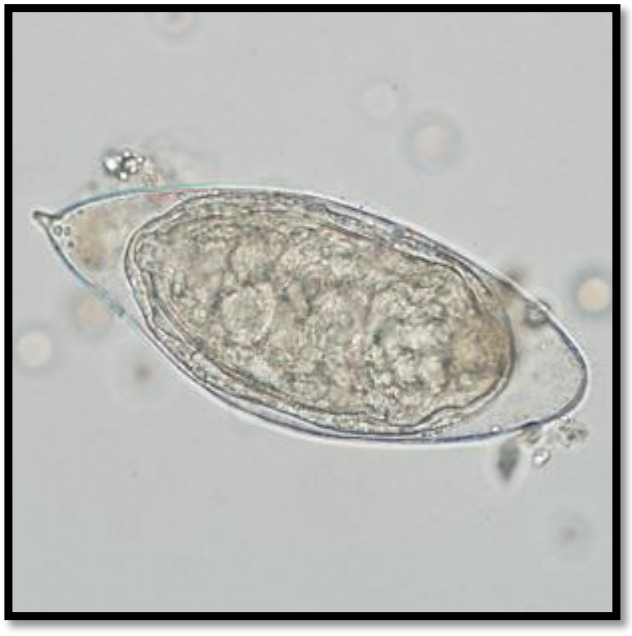
9.0 Procedure for Lower Respiratory Specimens

Sputum should be examined as a wet mount (saline or iodine), using low and high dry power (10X and 40X objectives). The specimen should not be concentrated before preparation of the wet mount. For thick sputum samples, an equal amount of 3% sodium hydroxide (or undiluted chlorine bleach) can be added; the specimen is thoroughly mixed and then centrifuged. NaOH should not be used for sample if *Entamoeba histolytica* is suspected. After centrifugation, the supernatant fluid is discarded and the sediment can be examined as a wet mount with saline or iodine.

Lower respiratory samples submitted for O & P should be examined for *Ascaris lumbricoides* larvae, *Strongyloides filariform* larvae, hookworm larvae, *Paragonimus westermani* ova, and *Echinococcus granulosus* hooklets, *Entamoeba histolytica. Cryptosporidium* spp. are rarely found in lower respiratory samples. If requested, *Cryptosporidium* may be ruled out by preparing a Modified Kinyouns smear.

10.0 Procedure for Urine

Urine submitted for examination to rule out *Schistosoma* species should be concentrated prior to microscopic examination. Ideally, at least 15 mL should be centrifuged at 500 x *g* for 5-10 min. Decant supernatant and examine a drop of sediment microscopically on low power (10X objective) for the presence of ova.



Egg of *S. haematobium* in a wet mount of a urine concentrate

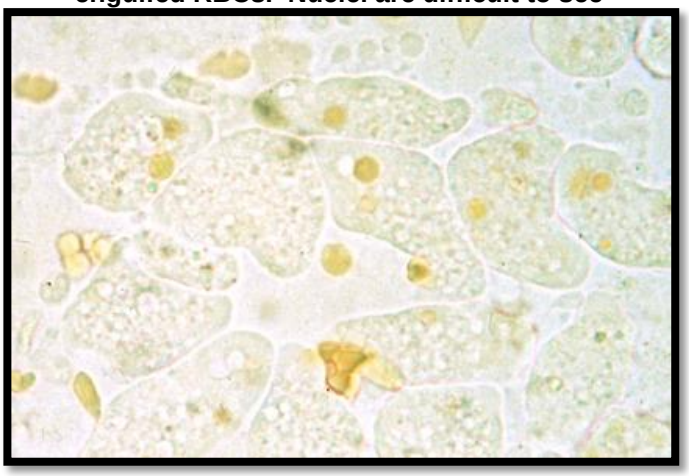
11.0 Procedure for Lung or Liver Abscesses

Examination of aspirates from the lung or liver abscesses may reveal trophozoites of *Entamoeba histolytica* or hydatid sand associated with *Echinococcus.*

Entamoeba histolytica

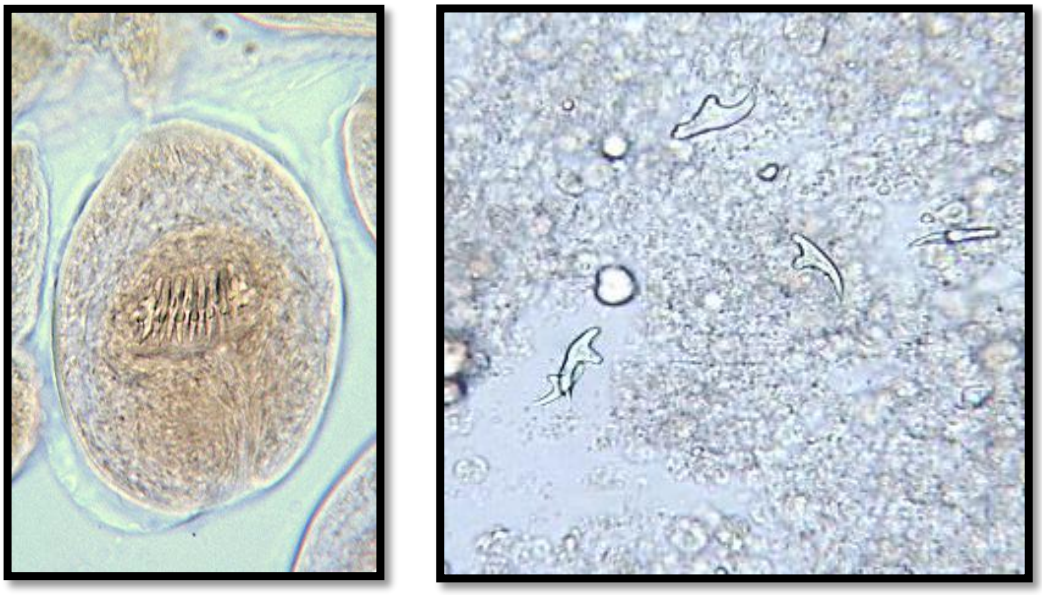
1. Perform a direct wet mount preparation without iodine to observe for motile trophozoites.
2. A permanent smear should also be prepared and stained with trichrome. The trichrome-stained smear should be reviewed on Rounds.

***Entamoeba histolytica* trophozoites with engulfed RBCs. Nuclei are difficult to see**



Echinococcus

1. If the aspirated material is fluid, centrifuge at 500 X *g* for 3 min.
2. If the aspirate is viscous or thick, the material can be mixed with saline or 10% KOH and then centrifuged at 500 X *g* for 3 min.
3. Carefully remove some of the sediment and prepare a wet mount.
4. Examine the material under low and high dry power. Hydatid sand consists of intact and degenerating scolices, hooklets, and calcareous corpuscles.
5. Hold the specimen for review on Rounds.



(L) Protoscolex of *Echinococcus granulosus* in a wet mount made from a liver aspirate.

(R) Hooklets in the sediment of hydatid fluid.

12.0 Reporting Results

12.1 Reporting in LIS

No ova or parasites seen

Since there are two results for each stool specimen, one for the concentrate and one for the trichrome, the first results should be entered and placed in "Hold" if no ova or parasites were seen. A header may be used on the initial entry to track what testing has been completed. When the results from the second test are available, the header should be removed and the results can be released as a consolidated final report.

Parasites seen

If ova or parasites are seen, the initial report can be released without any holds. However, no header should be used, and the report should only be modified if additional ova or parasites are seen during the second examination. The final report will contain one set of results and no headers for the trichrome or concentrate. This allows for one consolidated report rather than duplicating findings and comments under two separate headers.

12.2 Commensal vs. Pathogenic Organisms

Report the presence of all parasites. Commensal organisms and *Giardia* should be confirmed by another tech with $\geq$ 5 years of experience. Other pathogenic organisms should be confirmed on Rounds with the supervisor, director, or designee.

1. Commensal organisms of the gastrointestinal tract include the following:
   - *Endolimax nana*
   - *Entamoeba coli*
   - *Entamoeba hartmanni*
   - *E. polecki,*
   - *Iodamoeba bütschlii*
2. For commensal organisms, add the comment **"This parasite is considered to be non-pathogenic but indicates the patient has Ingested fecally contaminated food or water. Additional samples should be submitted for Ova and Parasite examination." [NPATOP]**

12.3 Non-Parasites

1. For organisms submitted that are not human parasites report, **"Specimen is negative for human parasites." [NOTPAR]**
2. For items submitted that do not contain an organism to identify report, **"No parasites present." [NOPAR]**

12.4 Fecal Leukocytes

If fecal leukocytes are present, report "fecal leukocytes seen." When fecal leukocytes are specifically requested also report absence of fecal leukocytes if applicable.

12.5 *Blastocystis* species

Add the comment, **"**There is controversy regarding the pathogenicity of *Blastocystis* species. It is commonly found in the intestinal tract but has been found to cause a self-limiting diarrhea. Studies suggest a correlation between infection density and symptoms. It is questionable whether treatment is necessary.**" [BLHOC]**

13.0 Quality Control & Quality Assurance

1. All staining dishes should remain covered to prevent evaporation of reagents.
2. All jars should be cleaned and refilled with new reagents as needed depending on the volume of slides stained. When the smear is thoroughly fixed and the staining procedure is performed correctly, the cytoplasm of protozoan trophozoites is blue-green, sometimes with a tinge of purple. Cysts tend to be slightly more purple. Nuclei and inclusions (chromatoidal bars, RBCs, bacteria, and Charcot Leyden crystals) are red, sometimes tinged with purple.

The background material usually stains green, providing a nice color contrast with the protozoa.

3. The positive control smear is made from known positive stool samples containing protozoan cysts or trophozoites. A control slide should be stained and examined weekly. The smear should be examined for proper staining. If staining is inadequate, the reagents should be replaced and a new positive control slide should be stained and read. Results of the control smear should be documented in the LIS.

14.0 Limitations

1. Inadequate Specimens
   Insufficient quantity for concentration procedure: Watery or scant specimens received in preservative may yield very little sediment after centrifugation. Add the following comment to the report: **A suboptimal amount of specimen was submitted and might lead to a false negative result. [QNSS]** If no material is observed after centrifugation, consult with the supervisor or charge tech.
2. Alternative Fixatives
   Fixatives other than Unifix™ and Total-Fix™ have not been validated for use. If specimens are received in an alternate preservative, a CRM case should be created. If the client does not want to recollect, PAML may be able to send the specimens to another reference lab for testing.
3. Pseudoparasites
   There are many items which may be encountered in fecal specimens that resemble or mimic parasites. Care should be taken to differentiate these items from pathogenic organisms. References that provide illustrations of some of the common pseudoparasites are available.
4. Demonstration of parasites from lung and liver abscesses can be difficult. Diagnosis is often made by serology rather than recovering the parasites from aspirated material.

15.0 Verification

Total-Fix preservative was evaluated in 2014. The manufacturer indicated that Unifix will be phased out and replaced with Total-Fix. Total-Fix is similar to Unifix but lacks PVA, making it compatible with EIA and molecular assays. Since fresh specimens are difficult to obtain, a limited verification study was performed to evaluate the performance of Total-Fix.

In the initial evaluation, live *Trichomonas vaginalis* was grown in culture and then preserved in both Unifix and Total-Fix. Smears were prepared from both samples and stained with trichrome staining reagents. When the smears were examined microscopically, no morphologic differences were observed.

Additional material was provided by the vendor for evaluation. Unstained smears prepared from specimens preserved in Total-Fix were obtained from Lynne Garcia's lab. These smears contained *Dientamoeba fragilis.* The smears were stained using our own staining reagents. The trophozoites observed in these smears presented a classic morphology and were easily recognizable.

The Parasep device was evaluated in June and July of 2015. Preserved specimens that were positive for a variety of parasites using conventional concentration methods were concentrated with the Parasep device. The Parasep concentrates were used to prepare trichrome and wet mounts for comparison to the conventional preparations. In all cases, the parasites recovered using the Parasep device were equivalent or better in morphology and quantity to those observed in the conventional preparations. The parasites examined include *Blastocystis* (4), *Chilomastix mesnili* cysts & trophs (1), *Dientamoeba fragilis* (1), *Endolimax nana* (2), *Entamoeba coli* cysts & trophs (3), *Giardia lamblia* cysts & trophs (3), *Cyclospora cayetanensis* (1), *Cryptosporidium* (1), *Ascaris lumbricoides* (1), *Diphyllobothrium latum* (1), *Hymenolepis nana* (1), *Strongyloides stercoralis* larvae (1), *Trichuris trichiura* (1), and *Taenia* spp. (1). In addition, 3 of the specimens examined contained fecal leukocytes in both conventional and Parasep preparations.

16.0 References

1. Package insert: Total-Fix™, Medical Chemical Corporation, Revision date: June 3, 2014.
2. Package insert: Unifix™, Medical Chemical Corporation, Revision date: April 23, 2009.
3. Garcia, Lynne, Diagnostic Medical Parasitology. 2007. 5th Ed.
4. Wheatley, W.B.: A Rapid Staining Procedure for Intestinal Amoeba and Flagellates. Am. J. Clin. Path., 21: 990-991, 1951.
5. Couturier, B.A., Jensen, R., Arias, N., Heffron, M., Gubler, E., Case, K., Gowans, J., and Couturier, M.R.: Clinical and analytical evaluation of a single vial stool collection device with formalin free 2 fixative for improved processing and comprehensive detection of gastrointestinal parasites. J. Clin. Microbiol. Accepted Manuscript posted online May 27, 2015
6. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L Landry, M.A. Pfaller. 2007. Manual of Clinical Microbiology, 9th ed., Vol. 1, ASM Press, Washington, D.C.
7. Tan KS. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. Clin Microbiol Rev 2008:639-65.

17.0 Document Control

Medical Director Approval: Reviewed by Dr. Schappert 03/10/2010

Microbiology Director Approval: Dr. Ann Robinson 10/14/2011

Microbiology Supervisor Reviews: Jerry Claridge 05/02/2002, 04/18/2003, 03/2004, 12/2005, 2/28/2006, 09/2006, 03/2007, 10/2008, 02/2009, 06/2011, 10/17/2011, 03/2013

Revisions:
10/28/2010 Updated concentration procedure for Unifix-preserved specimens to avoid formalin-Unifix precipitation. 10/14/2011 Added details for urine specimen collected for Schistosomes, added details for preparing trichrome smears with the appropriate thickness, added procedure for processing and examining lower respiratory and urine specimens, added details for reporting commensal organisms with comment, added verbiage for reporting items submitted that are not parasites, changed Blastocystis hominis to Blastocystis spp., added criteria for quantitating Blastocystis, modified clinical significance comment for Blastocystis, added detail for determining integrity of staining reagents and examining weekly control smear, and added limitations for scant specimens and potential for pseudoparasites. 10/18/2011 Removed PVA as an acceptable fixative. 11/16/2011 Added *Giardia* to list of parasites that can be confirmed by a senior tech. 3/21/14 Added comment: A suboptimal amount of specimen was submitted and might lead to a false negative result [QNSS]. 9/2/2014 Protocol was updated for Total-Fix. Permanent smears made from sediment after first spin. Reporting protocol updated to eliminate the use of headers for trichrome and concentrate. Preliminary results are entered and held. Results entered once and released after both tests have been performed. 9/23/14 Removed quantitation for fecal leukocytes. 9/26/14 Removed quantitation for *Blastocystis* species. Removed, “although metronidazole has been reported to be effective” from the BLHOC comment. Added details for holding and releasing positive and negative results. 7/15/15 Added procedure for testing liver and lung abscess specimens. Removed protocol for ethyl acetate sedimentation and added Parasep concentration method.