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| Ova & Parasite Exam |
| **Purpose** | This procedure provides information for processing and reading of ova and parasite exams. |
| **Principal** | Diagnosis of intestinal parasitic disease is confirmed by recovery and identification of helminth eggs and larvae, or protozoan trophozoites and cysts in the clinical laboratory. Timely collection and transportation of fresh stool to the laboratory cannot always be insured. If the specimen cannot be submitted to the laboratory within two hours, it should be placed in preservative, preferably the two vial system, which includes PVA and 10% formalin. This method will preserve cysts, trophozooites, eggs and larvae.A concentration method and permanent slide will be done on all specimens. |
| **Policy Statements** |  Microbiologists/virologists who perform O&P exams.  |
| **Test Code** | OAP |
| **Materials** | **Reagents** | **Supplies** | **Equipment** |
|  | * 0.85% Saline
* 10% Formalin
* Dobell & O’Connor Iodine dropper
* Trichrome stain
* Glacial Acetic acid
* Water
* 95% ethyl alcohol
* 100% ethyl alcohol
* Americlear
* Mounting Medium
* Para-Pak Zn-PVA/Formalin Kit

Para-Pak® Con-Trate® System(product # 960050)* Con-Trate Filter
* Con-Trate disposable tube and cap
* Reagent A (MucoPenX)
* Reagent B (ethyl acetate)
 | * Disposable gloves
* Disposable transfer pipettes
* Wooden applicator sticks
* Paper towels
* Plain glass slides
* Poly-L-Lysine adhesive coated slides
* 22x22 coverslips
* 24x50 coverslips
* Cotton-tipped applicator sticks
 | * Centrifuge -Baxter Megafuge 1.0 —display G#, by pressing and holding the speed/rcf set button---
* Timer
* Microscope
* Micrometer
* 35º C ambient air incubator
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| Sample | 1. Acceptable specimens
2. Two to three grams of liquid, semi-soft, or solid stool.
3. Stool aspirate
4. Duodenal aspirate
5. Urine for schistosomes
6. Formalin and PVA vials with sufficient stool added to each to bring the liquid level up to the “Fill to Here” line.
7. Sputum
8. SDES codes/Specimen type
* STO – stool
* STO-ASP – stool aspirate
* DA – duodenal aspirate
* SPU- sputum
* UR; VOID; CATH -- urine
1. Specimen Collection and Transport
* Refer to Lab Test Directory – Ova and Parasite Examination, Stool
1. Specimen assessment
* Refer to the Sample Rejection section of Lab Test Directory – Ova and Parasite Examination, Stool
 | [Lab Test Directory – Ova and Parasite Examination](https://www.childrensmn.org/References/Lab/microbioviral/ova-and-parasite-examination-stool.pdf)[Lab Test Directory – Ova and Parasite Exam, Aspirated Specimen](https://www.childrensmn.org/References/Lab/microbioviral/ova-and-parasite-exam-aspirated-specimen.pdf) (for Duodenal Aspirates, Sigmoidoscopy) |
| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies:1. [Biohazard Containment](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.1%20Biohazard%20Containment.docx)
2. [Safety in the Microbiology/Virology Laboratory](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.2%20Safety%20in%20the%20Microbiology%20Lab.docx)
* [Biohazardous Spills](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.4%20Biohazardous%20Spills.docx)
1. Formalin is a potential cancer hazard.
2. Zn-PVA is flammable.
3. CON-Trate Reagent B (Ethyl Acetate) is flammable.
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| **Quality Control** | 1. Perform Trichrome QC weekly, (after trichrome stain has been changed). Use a known positive *Giardia* PVA smear.
2. Record Trichrome QC results on QC sheet on desk 3.
3. Perform Iodine Preparation QC with each new lot number using Formalin preserved specimen containing Giardia cysts.
4. If there is a QC failure, document observation, notify supervisor.
5. The microscope must be calibrated and calibration factors posted by microscope.
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| **Sample Preparation** | Unpreserved stool--Formalin preservation1. Prepare a 1:3 to 1:6 dilution of stool to formalin using 5-10 grams of stool (minimum of 3 grams).
2. Examine the consistency of the stool.
3. Use the higher dilution if the stool is solid.
4. Use the lesser dilution if the stool is liquid.
5. Use a sterile container, (white flat bottom 20mL container or 15 mL centrifuge tube).
6. 5 grams stool to 10-20 mL 10% formalin
7. 3 grams stool to 6-12 mL10% formalin
8. Mix contents thoroughly, breaking up any lumps. Shaking for 1 min is usually sufficient.
9. Allow standing at RT for 30 min to for adequate fixation.

--PVA preservation 1. Place 1 drop of PVA on two Poly-L-Lysine adhesive coated slides, add and a mix a small amount of specimen into the PVA.
2. Using a wooden applicator stick, spread over approximately 1/3 of the slide, making “hills and valleys”, to create thick and thin sections for microscopic examination.
3. Allow drying for several hours in a 37º C incubator or overnight at room temperature.
4. **Para-Pak® Formalin transport system**
5. Fill Formalin and PVA vials with sufficient stool added to each vial to bring the liquid level up to the “Fill to Here” line.
6. Mix contents thoroughly. Shaking for 1 min is usually sufficient.
7. Allow standing at RT for 30 min for adequate fixation.
8. Using a Sunquest label, attach patient’s accession number to a treated slide.
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| **Storage** | 1. The specimen/preservative mixture must be stored for a minimum of 30 minutes after collection for adequate fixation. Mix contents thoroughly.
2. Preserved specimens should be maintained at room temperature.
3. Para-Pak Concentration Kit and Zn-PVA/Formalin Vials are stored at room temperature.
4. Iodine droppers must be stored away from exposure to light.
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| **Procedure** | **Fecal Con-Trate Concentration** 1. Formalin Vial
2. Add 4 drops of Reagent A to the specimen in the Formalin vial.
3. Cap and mix thoroughly by shaking the vial.
4. Insert a Con-Trate filter into the top of the 15ml centrifuge tube provided.
5. Pour fecal suspension through filter into the centrifuge tube. Three milliliters (3 mL) in the tube is sufficient unless the suspension is thin. DO NOT force the specimen through.
6. Discard filter. Add saline to the 12 mL mark, mix contents, cap tube and centrifuge for 10 minutes at 2000 rpm. **Note:** To determine RPM vs. relative centrifugal force (rcf or xg), hold the RPM set button down on centrifuge until rcf reading displays.
7. Decant supernatant and retain sediment. Sediment of 1.0 mL is optimal.
8. Re-suspend sediment in 10 mL of 10% formalin.
9. Add 3 mL of reagent B, cap tube, and shake for 30 seconds. Invert the tube while shaking. CAUTION: Pressure may build in the tube while shaking. Release by slowly opening the cap away from your person.
10. Centrifuge for 10 minutes at 2000 rpm. Examination of the tube after centrifugation should reveal four distinct layers from the top down:
* Layer consisting of Reagent B
* Plug of fecal debris
* Discolored aqueous layer
* Sediment layer, containing the parasites. The final sediment should be 0.25-0.5 mL.
1. Hold the tube at a 45-degree angle and free the plug of debris by ringing with a wooden applicator stick. Decant the upper layers into the Ethyl acetate waste container, leaving the sediment.
2. While holding the tube down, clean the sides of the tube with cotton tipped swab.
3. Prepare two slides, saline mount, and iodine mount for examination.
4. Both entire coverslips (iodine and saline) should be systematically examined under low (10X) power (to scan for suspicious objects) and high (40X) powers (to confirm or rule out) for detection of eggs, cysts, and trophozooites.

**Permanent Stain:** The detection and correct identification of many intestinal protozoa frequently depend on the examination of the permanent stained smear with the oil immersion lens (100X). These slides provide a permanent record of protozoan organisms identified. Examine at least 300 oil immersion fields using the 100X oil immersion objective. 1. Preserved Specimen Technique

Make 2 slides. Use the spoon inside the Zn-PVA vial (grey-topped vial); place 1-2 drops of specimen onto each of two Poly-L-Lysine adhesive coated slides.1. Using a wooden applicator stick, spread over approximately 1/3 of the slide, making “hills and valleys”, to create thick and thin sections for microscopic examination.
2. Allow the slides to dry for several hours in a 37º C incubator or overnight at room temperature.
3. Fresh Specimen Technique (must be done at time of specimen accession):
4. Make 2 slides. Place 1 drop of PVA on two Poly-L-Lysine adhesive coated slides, mix a small amount of specimen into the PVA and spread over approximately 1/3 of the slide, making “hills and valleys”, to create thick and thin sections for microscopic examination.
5. Allow the slides to dry for several hours in a 37º C incubator or overnight at room temperature.
6. Trichrome Staining Procedure:
7. Once Zn-PVA prepared slides have dried completely, staining may begin by placing the slides directly into the trichrome stain.
8. Trichrome Stain 6-8 minutes
9. 95% Ethyl Alcohol, acidified 2 quick dips
10. 95% Ethyl Alcohol (1) Dip twice
11. 95% Ethyl Alcohol (2) 5 minutes
12. 100% Ethyl Alcohol 10 minutes
13. Americlear 10 minutes
14. Mount with a 24x50 coverslip using mounting medium. Allow to set, in incubator, for a minimum of 1 hour (maximum 2 hours). Do not incubate overnight, this will prevent bubble formation.
15. Evaluate the general nature of the smear under low power. If the slide appears unsatisfactory, stain the second slide or prepare another slide from the PVA vial.
	1. In the final stages of dehydration keep the 100% ethanol and the Americlear as free from water as possible. Coplin jars must have tight fitting caps to prevent both evaporation of reagents and absorption of moisture. If the Americlear becomes cloudy after the addition of slides from the 100% ethanol, return the slides to fresh 100% ethanol and replace the Americlear.
	2. If the smear peels or flakes off, the specimen may have been inadequately dried on the slide, or the slide may have been greasy.
16. Exam:

a. When using the microscope to view permanent stained smears, make sure the light is very bright. b. Examine at least 300 oil immersion fields using the 100X oil immersion objective before indicating that the permanent stained smear is negative. 1. Stain Maintenance:

a. Change trichrome stain every two weeks.b. Change reagents, alcohols and Americlear weekly.1. Preparation:
2. Acid Alcohol for trichrome stain
3. 45 mL 95% Ethyl Alcohol
4. 2.5 mL water
5. 0.25 mL Glacial Acetic Acid

**Urine for Schistosome exam**1. *Schistosoma haematobium* eggs are usually detected in the urine. **Peak egg excretion occurs between noon and 3 p.m. Samples should be collected during this time. Transport directly to the laboratory (≤15 min) since specimens must be examined within 1 hour of collection.**1. Several (5) 15 ml aliquots of urine should be examined after centrifugation at 500 X G (1700 rpm) for 10 minutes. Pour off the supernatant and examine at least ten coverslip preps of sediment looking for Schistosome eggs.
2. The entire coverslip should be systematically examined under low (10X) and at least half of the coverslip at high (40X) powers for detection of eggs. Note live or mature miracidia in active infections.
3. If eggs are present, report the genus and species and whether the eggs are viable or non-viable.

Example: 1. Schistosoma *haematobium* eggs present (viable eggs seen). 2. *Schistosoma haematobium* eggs present (nonviable, eggshells only).**Sputum for Parasite Exam:** Parasites that can be detected include Entamoeba histolytica, Paragonimus spp., Strongyloides stercoralis, Ascaris lumbricoides, and hookworm. 1. Mix 1 ml of sputum with 8 drops of Reagent A. 1. Incubate at room temperature for 15 minutes.
2. Add 2 ml of saline.
3. Centrifuge for 5 minutes at 1,000-x g (2300rpm).
4. Decant supernatant and use sediment to prepare wet mounts for microscopic examination, and smears for trichrome permanent stain.
5. **Direct Wet mounts**: **Must be examined within 1 hour of collection**.

Prepare two slides, saline mount, and iodine mount.1. Mix a small amount of sputum sediment with a drop of 0.85% saline on a glass slide and cover with a 22x22 coverslip.
2. Mix a second drop of sputum sediment with Dobell and O’Connor iodine and coverslip.
3. If present, the blood or mucus portions of the specimen should always used for the direct mounts
4. Both entire coverslips (iodine and saline) should be systematically examined under low (10X) power (to scan for suspicious objects) and high (40X) power for detection of eggs, cysts, and trophozooites. Mobility of trophozooites should also be noted in fresh specimens.
5. **Trichrome Permanent stain:** Make 2 slides by mixing a drop of sediment and a drop of PVA fixative on blue adhesive slides. When slides are dry, stain with trichrome stain, as in Permanent Stain section.
6. **Preserve remaining specimen** by adding formalin in 1:3 ratio (sputum: formalin).
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| **Limitations** | 1. Refer to the policy *Specimen Rejection Criteria for Microbiology/Virology* for monitoring the acceptability of specimens for testing. Unless guidelines for delivery times are followed and inappropriate specimens are rejected, the laboratory results may be incorrect.
2. Many organisms do not appear in stool specimens in consistent numbers on a daily basis; few or no parasites may be passed on one day, with many passed the next day. No more than one specimen should be submitted per day.
3. Parasite exams on patients hospitalized >3 days are not productive and should not be ordered unless special circumstances exist.
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| **Method Performance Specifications** | 1. If only a formalin vial is received, the doctor must be called and testing cancelled. If they are interested in *Giardia or Cryptosporidium*, a CRID can be offered.
2. If only a PVA vial is received, a concentrate and permanent smear can be performed from this vial.
3. If a SAF or ECOFIX vial is received, it can be processed for concentrate and permanent smear as described above. Be sure to use the Poly-L-Lysine adhesive coated slides.
4. Initial Micrometer calibration: Use micrometer to measure the planar dimension in a microscope field. To measure the length of an object, note the number of ocular divisions spanned by the object. Then multiply by the conversion factor for the magnification used. The conversion factor is different at each magnification. Use table below:

Example: Using the Olympus scope, you measure 3 divisions with the 40x objective, multiply 3 x 2.3µm = 6.9µm. Round to 7µm.

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| --- | --- | --- | --- | --- | --- |
| Scope Brand | Date | BioMedAssetNumber | Location | Power of Objective |  |
|  |  | 10x | 20x | 40x | 100x |  |
| Nikon | 2002 | 008294 | MPLS | 10.0 µ | 5.0µ | 2.5µ | 1.0µ |
| Nikon | 2002 | 008422 | MPLS | 10.0 µ | 5.0µ | 2.5µ | 1.0µ |
| Olympus | 2019 | 032761 | MPLS | 9.9µ | 4.8µ | 2.3µ | 0.76µ |  |

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| **Result Reporting** | 1. Record results and workups in Sunquest Microbiology Result Entry in the Culture Entry tab.
2. **Negative:** No Ova and Parasites Seen (Sunquest MO code: **NOPS**)
3. **Positive:** Report the parasite and stage (trophozoite or cyst). If the MO code is unknown, do a keyword look-up by typing a semicolon in the result box and click on the **ellipsis** button. Type in a partial/entire word as follows: HYMENOLEPSIS. Search on **Description**. Select the desired code by highlighting.

Search ValueText Code: HYMENSearch Option ○ Code ◙ DescriptionMatched on Code OptionHYMENOLEPS... HDO HYMENOLEPSIS DIMINUTA EGGSHYMENOLEPS... HNAN HYMENOLEPSIS NANA EGGSObservations: 1. HYMENOLEPSIS NANA EGGS Workups: Workup #1 Workup Comments Med : FORM DIR : Desc : PARA CONC : HNAN ID : PARA TRS : COM : ;Measures 40 Workup #2 Workup Comments Med : PVA DIR : Desc : PARA CONC : ID : PARA TRS : NOPS1. Quantitation:
2. Quantitative *Blastocystis hominis* (few, moderate, many). Do not quantitate other protozoa or helminths.
3. Quantitate human cells (WBC’S, RBCs and eosinophils).
4. Quantitate artifacts like Charcot-Leyden crystals.

Example: *Blastocystis hominis,* cells and artifacts PVA smears Wet preps No. per 10 fields No. per 10 fields (oil 100X) (40X)FEW <=2 <=2 MODERATE 3 – 9 3 – 9 MANY >=10 >=10 1. If additional information is available after the exam has been finalized, remove the final status and send out a supplementary report using the code SRPT in SREQ or CULTURE RESULTS. Refinal the culture when identifications and/or testing is complete.
2. If an exam requires a correction, the code **CORR** (corrected report) must be used in CULTURE RESULTS. Refer to the procedure *CORRECTED LABORATORY REPORTS.*
 |
| **References** | 1. Garcia, L. S., *Diagnostic Medical Parasitology*, 4th Ed., 2001, ASM Press, Washington, DC.
2. Shimizu, R.Y.,Parasitology Section*, Clinical Microbiology Procedures Handbook,* L. Garcia (Ed) volume 2, 4th edition, 2016. ASM Press
3. *Laboratory Procedure for the Diagnosis of Intestinal Parasites*, U.S. Dept. of Health and Human Services, PHS, HHS Publication No. (CDC) 80-8282
4. Meridian Con-Trate System package insert, Bioscience, Inc., 1994, Rev 5/2003 Rev. 7/2014.
5. Forbes, B.A., et al., Bailey & Scott’s *Diagnostic Microbiology***,**, 12th edition, 2007, Mosby, Inc., St. Louis, MO., pg.543-627
6. Murray, P.R., et al, *Manual of Clinical Microbiology*, 9th edition, 2007, ASM press, Washington DC, pages132-149.
7. Meridian Para-Pak Zn-PVA and Formalin Systems package insert, Bioscience, Inc., 1985, Rev 5/2014.
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| **Appendices** | WORK LABEL MEDIA-FORM DEFINITIONBATTERY: OAPSPEC MEDIA FORM, PVA |
| **Training Plan/****Competency** | 1. Employee must read the procedure. Atlases, Kodachrome slides and survey materials are available for training.
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | 1. Direct observation
2. CAP Surveys, performed at least annually.
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1.0 | Pat Ackerman | 1975 | Initial Version |
| 1.1 | Maria Dunphy | 1984 |  |
| 1.2 | Maria Dunphy | 01/31/1992 |  |
|  | 1.3 | Peggy Winston | 09/16/2003 | . Modified SRPT and CORR statements. |  |  |
| 1.4 | Peggy Winston | 04/29/2007 | Updated Sunquest 6.2 reporting informationAmericlear replaces Citrisolv, Dobell & O’Connor Iodine dropper replaces Lugol’s iodine |
| 1.5 | Jessica Craig | 06/28/2010 | Updated into online format. |
| 1.6 | Eileen Brinkman | 05/05/2011 | Added sputum O&P to procedure, added rcf vs rpm on centrifuge, and added Iodine QC. |
| 1.7 | Eileen Brinkman | 09/04/2013 | Removed quantization of parasites and cancellation of Formalin vial only submission. |
| 2 | Becky Carlson | 4/26/2015 | Re-numbered from MC 501 for CMS load |
|  | 2.1 | Becky Carlson | 7/1/2015 | Added Sample Preparation section. |
|  | 2.2 | Becky Carlson | 9/19/2016 | Micrometer calibration data expanded to include dates and asset numbers of microscopes. |
|  | 3 | Becky Carlson | 11/15/2016 | Removed Direct Wet Mount procedural steps. Direct examination for motility no longer performed when fresh stool or fresh stool aspirates are submitted. |
|  | 3.1 | Becky Carlson | 3/14/2017 | Added Sputum Direct Wet Mount procedural steps.  |
|  | 3.2 | Susan DeMeyere | 5/26/2017 | Removed Cytoseal 60, replaced with mounting medium.  |
|  | 4 | Susan DeMeyere | 6/17/2019 | Added new scope calibration and instructions Remove reporting of yeast.  |