

FECAL CONCENTRATION (PARA-SED, 50 ML)

Preanalytical Considerations

I. PRINCIPLE

PARA-SED™ is a closed concentration system for the recovery of eggs, larvae, and protozoa from preserved fecal specimens. PARA-SED is designed to be used with 5% or 10% formalin, sodium acetate-acetic acid-formalin (SAF), polyvinyl alcohol (PVA), and Z-PVA preserved material. When used with Para-Fix™ collection vials, PARA-SED provides a convenient and reproducible method for detecting parasites even when present in very low numbers. The diagnosis of parasitic infection is confirmed by the recovery of helminth eggs and larvae, protozoan trophozoites and cysts, coccidian oocysts, and microsporidian spores. A concentration procedure should be performed as a routine part of a complete examination for parasites. Concentration procedures permit the detection of organisms present in small numbers that may be missed using only the direct wet mount. Organisms that can generally be identified using a concentration procedure include: helminth eggs and larvae; cysts of *Giardia lamblia*, *Entamoeba histolytica-E. dispar*, *Entamoeba coli*, and *Iodamoeba bütschlii*; and oocysts of *Isospora belli*. The identification of other protozoa should be considered tentative and should be confirmed with a permanent stain smear or special stains (coccidia, microsporidia).

II. SPECIMENS

The specimen can be fresh stool or stool that has been preserved in 5 or 10% formalin, SAF, PVA, Z-PVA, or some of the other single-vial system fixatives. For the concentration of fresh material collected in the Para-Fix clean vial, add 15 ml of 5% or 10% formalin, SAF, PVA, Z-PVA, or some of the other single-vial system fixatives to a 3-5 g of sample. Mix well, and allow to stand for at least 30 min before processing.

III. MATERIALS

A. Kit Contents:

- a. 50 Concentrator Units
- b. 50 50 ml Centrifuge Tubes (Vials)
- c. 50 Caps
- d. Tray
- e. 1 Bottle Surfactant
- f. 1 Instruction Sheet

B. Supplies and Equipment Not Provided:

- a. Cotton Tip Applicators
- b. Centrifuge
- c. Transfer Pipettes
- d. Microscope Slides and Coverslips
- e. Microscope

- f. Physiological Saline (0.85% NaCl)
- g. Ethyl Acetate
- h. Applicator Sticks
- i. Lugol's or D'Antoni's Iodine

C. Equipment Required

- a. Binocular microscope with 10X, 40X, and 100X objectives (or the approximate magnifications for low power, high dry power, and oil immersion examination).
- b. Oculars should be 10X. Some workers prefer 5X; however, overall smaller magnification may make final organism identifications more difficult.
- c. Centrifuge that accepts 50 ml tubes (Tabletop centrifuge may or may not be large enough)

Analytical Considerations

IV. QUALITY CONTROL

- A. Check the reagents each time they are used. The formalin and saline should appear clear, without any visible contamination.
- B. When stored at room temperature, the product is stable for two years from the date of manufacture. The user should verify this date and should examine the concentrator unit for cracks, and the surfactant for bacterial or fungal contamination.
- C. The microscope should be calibrated and the objectives and oculars used for the calibration procedure should be used for all measurements calculated using that particular microscope. Post the calibration factors for all objectives on the microscope for easy access (multiplication factors can be pasted right on the body of the microscope). Although there is not universal agreement, the microscope should probably be recalibrated once each year. This recommendation should be considered with heavy use or if the microscope has been bumped or moved multiple times. If the microscope does not receive heavy use, then recalibration is not required on a yearly basis.
- D. Concentrate known positive specimens and verify organism recovery at least quarterly and particularly after the centrifuge has been recalibrated.
- E. Record all QC results; the laboratory should also have an action plan for "out of control" results.

V. PROCEDURE

- A. *Wear gloves when performing this procedure.*
- B. Remove the cap from the specimen vial and add 8-10 drops of surfactant. Recap the vial, making sure the lid is securely fastened.

- C. Mix the contents of the vial by shaking vigorously or vortexing for 30 seconds.
- D. With the 50 ml centrifuge vial still loosely attached to the filter unit (loose attachment will facilitate the release of air pressure during use), insert the open end of the filter unit into the specimen vial until the sealing ring is firmly seated. Tighten the 50 ml centrifuge vial onto the filter unit.
- E. Invert the tube, and filter the specimen through the mesh into the 50 ml centrifuge tube. If the flow does not start immediately, or the specimen is thick, the flow may be initiated by sharply tapping the 50 ml centrifuge tube on a counter top.
- F. After filtration is complete, tap the 50 ml centrifuge tube on the counter top two or three times to insure that all the fluid has drained into the tube. Tilt the filter unit at a slight angle. Unscrew the concentrator unit and specimen vial and discard using established laboratory procedures for fecal specimens.
- G. Add 5% or 10% formalin, saline 0.85% NaCl* or SAF to bring the level of the filtered material to the red line on the PARA-SED centrifuge tube.
- H. Add 5 ml of ethyl acetate (or other ether substitute) to the 50 ml centrifuge tube. Recap the tube with the cap provided with the kit. Do not use the specimen vial cap; it will not fit.
- I. Shake vigorously for 30 s. If diethyl ether is used (not recommended) pressure may build up in the vial during shaking, and the cap should be carefully loosened after shaking to release the pressure, and then retightened.
- J. Centrifuge at 500 x g (1800 – 2500 rpm) for 10 min.
- K. Carefully remove the cap. The resulting solution should have four layers: Top: ethyl acetate or ethyl ether; Second: debris plug; Third: saline*; and Fourth: fecal sediment.
- L. Ring the debris layer with an applicator stick to loosen the debris.
- M. Invert the tube to pour off the supernatant fluid and debris layer.
- N. While the tube is still inverted, wipe the sides of the tube with one or two cotton-tipped applicators to remove ethyl acetate or debris left behind. Failure to remove the excess ethyl acetate may result in the formation of solvent bubbles in the wet mount. The sediment at the bottom of the vial will contain the parasites.
- O. Resuspend the pellet at the bottom of the tube with 0.85% NaCl*. If using SAF, do not resuspend the sediment. Remove some sediment and prepare smears for permanent staining prior to resuspension of the remaining sediment for wet preparation examination. If smears will be prepared for special staining (coccidia or the microsporidia), some of the remaining sediment can be used for making the smears. However, it is better to prepare these smears prior to resuspending the sediment for wet preparation examination.
- P. To prepare the wet mount, draw a sample from the resuspended fecal

- sediment with a capillary or transfer pipette. Place one or two drops on a microscope slide and cover with a coverslip. Examine immediately.
- Q. If an iodine mount is preferred, place one drop of Lugol's or D'Antoni's iodine on a slide to which is added one drop of the resuspended fecal material. Place a coverslip on the slide and examine immediately.
 - R. Systematically scan with the 10x objective. The entire coverslip area should be examined.
 - S. If you see something suspicious, use the 40x objective for more-detailed study. At least one-third of the coverslip should be examined with the 40x objective, even if nothing suspicious has been seen. As in the direct wet smear, iodine can be added to enhance morphological detail.

* 5% or 10% formalin, or SAF may be used in place of 0.85% NaCl.

VI. RESULTS

- A. Protozoan trophozoites and/or cysts, *Isospora belli* oocysts, and helminth eggs and larvae may be seen and identified. Protozoan trophozoites are less likely to be seen, especially when concentrated from 5% or 10% formalin. Trophozoites can be seen in the sediment when concentrated from SAF, although certainly the morphology is not as clearly seen as in the cyst forms.

Postanalytical Considerations

VII. REPORTING RESULTS

- A. Report the organism and stage (do not use abbreviations)
Examples (Stool Specimens): You may or may not be able to identify protozoan cysts to the species level (depending on the clarity of the morphology).
Example (positive report): *Entamoeba hartmanni* cysts present
Example (positive report): *Ascaris lumbricoides* eggs present
- B. You may also see artifacts and/or other structures. Report them.
Example (positive report): Few Charcot-Leyden crystals present.
Example (positive report): Moderate PMNs present

VIII. PROCEDURE NOTES

- A. Tap water may be substituted for 0.85% NaCl throughout this procedure, although the addition of water to fresh stool will cause *Blastocystis hominis* cyst forms to rupture. In addition to the original 10% formalin fixation, some workers prefer to use 10% formalin for all the rinses throughout the procedure.
- B. Ethyl acetate is widely recommended as a substitute for ether. It can be used the same way in the procedure and is much safer. Hemo-De can also be used. Use these agents in a well-ventilated area. Keep

away from direct flame. Avoid contact of the solution with skin and eyes. Should contact occur, flush with running water. Avoid breathing fumes.

- C. After the plug of debris is rimmed and excess fluid is decanted, while the tube is still upside down, swab the sides of the tube with a cotton-tipped applicator stick to remove excess ethyl acetate. This is particularly important if you are working with plastic centrifuge tubes/vials. If the sediment is too dry after the tube has been swabbed, add several drops of saline before preparing the wet mount for examination.
- D. If you have excess ethyl acetate in the wet mount of the sediment prepared for examination, bubbles will obscure the material you are trying to see.
- E. Too much or too little sediment will result in an ineffective concentration.
- F. Let the centrifuge reach the recommended speed before you begin to monitor centrifugation time. If the centrifugation time at the proper speed is reduced, some organisms (*Cryptosporidium* oocysts, microsporidian spores) may not be recovered in the sediment.

IX. LIMITATIONS OF THE PROCEDURE

- A. Results obtained with wet mounts should usually be confirmed by permanent stained smears. Some protozoa are very small and difficult to identify to the species level by direct wet mounts alone.
- B. Confirmation is particularly important in the case of *Entamoeba histolytica*/*E. dispar* versus *Entamoeba coli*.
- C. Certain organisms, such as *G. lamblia*, hookworm eggs, and occasionally *Trichuris* eggs, may not concentrate as well from PVA-preserved specimens as they do from those preserved in formalin. However, if there are enough *G. lamblia* organisms present to concentrate from formalin, then PVA should contain enough for detection on the permanent stained smear. In clinically important infections, the number of helminth eggs present would ensure detection regardless of the type of preservative used. Also, the morphology of *Strongyloides stercoralis* larvae is not as clear from PVA as from specimens fixed in formalin.
- D. For unknown reasons, *Isospora belli* oocysts concentrated from PVA-preserved specimens are routinely missed in the concentrate sediment.
- E. At the recommended centrifugation speed and time in this procedure, there is anecdotal evidence to strongly indicate *Cryptosporidium* oocysts and microsporidian spores should be recovered if present in the specimen. *The current recommendation is centrifugation at 500 X g for a minimum of 10 min for the recovery of coccidia and microsporidia.*

SUPPLEMENTAL READING

1. **ASMT, 1978.** Recommended procedures for the examination of clinical specimens submitted for the diagnosis of parasitic infections. *Am. J. Med. Technol.* **44**:1101-1106.
2. **Garcia, L.S.** 2001. *Diagnostic Medical Parasitology*, ed 4., ASM Press, Washington, D.C.
3. **Garcia, L.S. and R.Y. Shimizu.** 1981. Comparison of clinical results for the use of ethyl acetate and diethyl ether in the formalin-ether sedimentation technique performed on polyvinyl alcohol preserved specimens. *J. Clin. Microbiol.* **13**:709-713.
4. **Melvin, D.M. and M.M. Brooke,** 1985. *Laboratory Procedures for the Diagnosis of Intestinal Parasites*, U.S. Department of Health, Education, and Welfare publication no., (CDC) 85:8282, U.S. Government Printing Office, Washington, D.C.
5. **Neimeister, R., et al.** 1987. Hemo-De as a substitute for ethyl acetate in formalin-ethyl acetate concentration technique. *J. Clin. Microbiol.* **25**:425-426.
6. **NCCLS.** 1997. Procedures for the recovery and identification of parasites from the intestinal tract, Approved Guideline, M28-A, National Committee for Clinical Laboratory Standards, Villanova, PA.
7. **Yang, J.,** and T. Scholten. 1977. A fixative for intestinal parasites permitting the use of concentration and permanent staining procedures. *Am. J. Clin. Pathol.*, 67:300-304.
8. **Young, K.H. et al.** 1979. Ethyl acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. *J. Clin. Microbiol.* **10**:852-853.

APPENDIX

Relevant products available from Medical Chemical Corporation:

REAGENT	CATALOG NUMBER	SIZE AND CATALOG NUMBER	
PARA-SED (Closed Concentration System)	695A	695A	50 ml conc kit 50 kits/cs
UNIFIX Vials (Fecal Preservative)	2804-05	2804-05	10 vials/pk 100 vials/cs
LV-PVA Vials (Fecal Preservative)	2802-05	2802-05	10 vials/pk 100 vials/cs
Z-PVA Vials (Fecal Preservative)	2803-05	2803-05	10 vials/pk 100 vials/cs
SAF Vials	574-05	574-05	10 vials/pk 100 vials/cs
Formalin 5% Vials	5753-05	5753-05	10 vials/pk 100 vials/cs
Formalin 10% Vials	575-05	575-05	10 vials/pk 100 vials/cs
C&S Vials (Holding Medium for Stool Culture)	2805-05	2805-05	10 vials/pk 100 vials/cs
Clean Vials (Stool Collection Vials)	310	310	10 vials/pk 100 vials/cs
Ethyl Acetate	4992	4992-16 oz 4992-1gal	16 oz 1 gal