PRINCIPLE
PARA-PRO® *fc50* is a system for the concentration of helminth eggs, larvae, protozoa, coccidian oocysts, and microsporidia spores from preserved (fixed) fecal specimens. PARA-PRO *fc50* is designed to be used with PROTO-FIX®, 10% formalin, or SAF. When used with fixative collection vials, PARA-PRO *fc50* will form a closed filtration system for the pre-filtration step of the concentration procedure. PARA-PRO *fc50* was designed to filter the entire specimen prior to reagent concentration steps.

The diagnosis of intestinal parasitic infections requires appropriate collection, transport, concentration, staining and identification of parasites from fecal specimens. A wide variety of sedimentation, concentration, and flotation methods have been described.

The PARA-PRO *fc50* filtration unit has a precision molded filtration screen, which allows helminth eggs, larvae and protozoan cysts to pass through the screen but retains the larger diameter particulate matter on the top. Most of the macroscopic fecal debris will be discarded with the funnel/filtration unit. The addition of the surfactant to preserved specimens can help reduce the adhesive forces of the mucus, break up the lumps within the sample and increase settling of the parasitic eggs.

The formalin-ether sedimentation technique (Ritchie et al.) has been modified to avoid the flammability, storage and disposal of ether. The modified procedure replaces the ether with ethyl acetate. This procedure can be performed on specimens that have been fixed in PROTO-FIX®, buffered formalin, SAF, or on fresh specimens where the recommended fixative has been added during the processing. Ethyl acetate is less flammable than ethyl ether and as such, is recommended as a replacement to ether. The ethyl acetate will dissolve the fat and aid in the separation of the fecal debris from a concentrated sediment containing parasites, if present in the sample.

SPECIMEN COLLECTION AND PREPARATION
1. Specimens preserved in PROTO-FIX, buffered formalin, or SAF may be processed with the PARA-PRO *fc50*. The specimen must be fixed for a minimum of 60 minutes to assure adequate fixation of the sample. The specimen should be stored at room temperature.
2. For optimum results, it is recommended that specimens be preserved at the time of collection. Unpreserved specimens delayed in transport may have limited diagnostic value. To fix unpreserved specimens, transfer 2-3 grams of unpreserved stool into 13–15 ml of one of the preservatives listed above. Mix stool with preservative thoroughly and break up any lumps or fecal masses. The stool/preservative mixture should stand for a minimum of 60 minutes for adequate fixation.
3. An appropriate clinical patient sample, collected, preserved/fixed, and transported properly is important to the recovery of helminth eggs, larvae, and protozoan cysts. Please refer to appropriate references for the collection and transport methods.
4. Always mix the sample well.
5. The appropriate volume of sample is 2–3 grams of fecal matter in 13–15 ml of fixative.

FOR IN VITRO DIAGNOSTIC USE ONLY.

REAGENTS AND MATERIALS
1. **Provided**
    a. PARA-PRO *fc50* filtration units
    b. 50 ml and 15 ml Centrifuge tubes & caps
    c. Triton X-100™

2. **Not Provided**
    a. Microscope
    b. Microscope slides and cover slips
    c. Centrifuge
    d. Cotton-tipped applicator sticks
    e. Pipettes
    f. PROTO-FIX (or other fixative)
    g. CONSED® Concentration Reagent or equivalent
    h. ethyl acetate or equivalent
    i. Physiological saline

3. **Storage:** Store at room temperature (15-30ºC). Avoid excessive heat and sunlight.

4. **Stability:** The PARA-PRO *fc50* filtration units are stable to the stated expiration date when stored at the required temperature.

CALIBRATION
N/A

QUALITY CONTROL
The PARA-PRO *fc50* should be examined for integrity of the device, i.e., the device should not be cracked, the filtration cone should have a consistent hole opening pattern (no blockage of filtration holes) and the airway holes at the top of the filtration cone should be free of residual plastic. Any device showing irregularities should not be used.

PROCEDURE

Specimen Processing:
Filtration

1. Thoroughly mix the specimen fixed in PROTO-FIX, buffered formalin, or SAF by shaking the specimen/fixative vial.
2. Add 4–5 drops of the Triton X-100 to the specimen/fixative vial (up to 8 drops may be added if the specimen is highly mucoid).
3. Re-cap the fixative vial and mix the contents thoroughly by shaking for 20 to 30 seconds.
4. Appropriately label one side of the 50 ml receiver centrifuge tube. With the 50 ml centrifuge tube attached to the PARA-PRO *fc50* filtration unit, insert the open end of the filtration unit into the fixative/specimen vial and twist slightly until the seal is tight. Tighten the 50 ml centrifuge tube onto the PARA-PRO *fc50* filtration unit.
5. Invert the tube, and filter the specimen through the PARA-PRO *fc50* filtration unit into the 50 ml centrifuge tube. If the flow does not begin immediately, sharply tap the 50 ml centrifuge tube on the counter top.
6. After the filtration is complete, tap the 50 ml centrifuge tube on the counter top 2-3 times. Tilt the filtration unit at a slight angle and unscrew the PARA-PRO *fc50* and specimen vial from the 50 ml centrifuge tube and discard into appropriate disposal container following established laboratory procedures for fixed fecal specimens.
7. Prepare the wet mount by placing 1 drop of the filtered specimen onto a clean glass slide. Add a drop of iodine solution (Lugol's Iodine or Dobell & O'Connor's Iodine) to the specimen, mix gently and coverslip. Examine the slide microscopically for ova, helminths and parasites. Consult appropriate references for the identification of ova and parasites.
8. The resulting filtered specimen can be further concentrated to improve recovery of ova and parasites. See below for the appropriate procedures for each fixative.
9. Refer to the Directions for Use for the specific fixative used regarding the staining of permanent mounts from the filtered specimen.

Concentration/Sedimentation Procedures:
PROTO-FIX Preserved Specimens
CONSED Concentration Procedure
NOTE: PROTO-FIX fixative is a clear, colorless one vial processing fixative for wet preparations, permanent stains, concentrations, DFA and EIA methodologies for fecal specimens used in the diagnosis of intestinal parasites. PROTO-FIX is a low alcohol, low viscosity fixative that contains no heavy metals, PVA or aniline dyes. The CONSED Concentration Reagent is recommended as it increases the recovery of ova, helminths and parasites. In addition, the PROTO-FIX / CONSED concentration procedure increases laboratory efficiencies and the diagnostic value of the permanent stain, as the permanent smear of specimens that have been fixed in PROTO-FIX can be performed from the CONSED concentrated pellet.

1. Pour 2 ml of the filtered specimen into a 15 ml centrifuge tube. To the 2 ml of the filtered PROTO-FIX fixed specimen, add 8 ml of the CONSED reagent and 4 ml of ethyl acetate (or replacement reagent) to the sample in the centrifuge tube. Cap the tube, invert the tube and shake vigorously for 30 seconds. Pressure will build up within the tube during shaking. To remove this pressure loosen the cap carefully, and then retighten the cap prior to centrifugation.
2. Place the capped centrifuge tubes into the centrifuge (with a free swinging head) and centrifuge for 10 minutes at 500–600 xg Following centrifugation, four layers will develop:
    a. A top layer of mostly ethyl acetate
    b. An interface layer of fatty fecal debris
    c. A lower solution layer
    d. A pellet/sediment layer
3. Holding the centrifuge tube in a vertical position, remove the cap, free the plug of debris from the sides of the tube by ringing the tube with a wooden applicator stick. Carefully pour the top three layers into an appropriate waste container. **NOTE:** If the pellet begins to break up, quickly upright the tube to save the pellet, then carefully aspirate any residual reagent off of the pellet with a pipette. While the tube is still tipped in the decanting position, use cotton-tipped swabs to remove remaining debris and ethyl acetate from the sides of the tube. Do not turn the tube upright until the sides of the tube have been thoroughly cleaned of the reagent solutions. If the sides are not cleaned thoroughly with the swab, lipid droplets can mix with the sediment pellet making the microscopic examination more difficult. Allowing excess ethyl acetate to run back into the pelleted sediment will result in a poor wet mount preparation due to the formation of solvent bubbles.
4. Add 3–6 drops (or an amount equal to the volume of the pellet) of PROTO-FIX. Using an applicator stick, thoroughly mix the PROTO-FIX with the pellet. **NOTE:** The smear for the permanent stain and slides for special stains can be made at this point in the procedure. See the section header "Preparing Slides for Smears", and "Miscellaneous Procedures" in the PROTO-FIX Directions For Use.
5. Prepare the wet mount by placing 1 drop of the pellet prepared in step #4 onto a clean glass slide. Add a drop of iodine solution (Lugol's Iodine or Dobell & O'Connor's Iodine) to the specimen, mix gently and coverslip. Examine the slide microscopically for ova, helminths and parasites. Consult appropriate references for the identification of ova and parasites.

Concentration/Sedimentation Procedures:
PROTO-FIX Preserved Specimens
Formalin/Ethyl Acetate Concentration Procedure
NOTE: The formalin/ethyl acetate concentration procedure is not the recommended for use with specimens fixed in PROTO-FIX. Using the formalin/ethyl acetate procedure will reduce the number and variety of parasites in the concentrated sample and will prevent the recovery of any trophozoites present. Using the PROTO-FIX/CONSED concentration procedure (listed previously) will significantly improve organism recovery in the concentrated sample and is strongly recommended.

1. Pour 3 ml of the filtered specimen into a 15 ml centrifuge tube. To the 3 ml of the filtered PROTO-FIX fixed specimen, add 7 ml of 10% buffered formalin and mix the specimen.
2. Add 4 ml of ethyl acetate (or replacement reagent) to the sample in the centrifuge tube. Cap the tube, invert the tube and shake vigorously for 30 seconds. Pressure will build up within the tube during shaking. To remove this pressure, loosen the cap carefully, and then retighten the cap prior to centrifugation.
3. Place the capped centrifuge tubes into the centrifuge (with a free swinging head) and centrifuge for 10 minutes at 500–600 xg. Following centrifugation, four layers will develop:
   a. A top layer of mostly ethyl acetate
   b. An interface layer of fatty fecal debris
   c. A lower solution layer
   d. A pellet/sediment layer
4. Holding the centrifuge tube in a vertical position, remove the cap, free the plug of debris from the sides of the tube by ringing the tube with a wooden applicator stick. Carefully pour the top three layers into an appropriate waste container. **NOTE:** If the pellet begins to break up, quickly upright the tube to save the pellet, then carefully aspirate any residual reagent off of the pellet with a pipette. While the tube is still tipped in the decanting position, use cotton-tipped swabs to remove remaining debris and ethyl acetate from the sides of the tube. Do not turn the tube upright until the sides of the tube have been thoroughly cleaned of the reagent solutions. If the sides are not cleaned thoroughly with the swab, lipid droplets can mix with the sediment pellet making the microscopic examination more difficult. Allowing excess ethyl acetate to run back into the pelleted sediment will result in a poor wet mount preparation due to the formation of solvent bubbles.
5. Add a few drops of PROTO-FIX to the pellet and mix well.
6. Prepare the wet mount by placing 1 drop of the sediment prepared in step #5 onto a clean glass slide. Add a drop of iodine solution (Lugol's Iodine or Dobell & O'Connor's Iodine) to the specimen, mix gently and coverslip. Examine the slide microscopically for ova, helminths and parasites. Consult appropriate references for the identification of ova and parasites.

Concentration/Sedimentation Procedures:
Formalin and SAF Preserved Specimens
Formalin / Ethyl Acetate Concentration Procedure

1. Add 10 ml of physiological saline to the filtered sample (in the 50 ml centrifuge tube) and centrifuge 500–600 xg for 10 minutes. Decant the supernatant, retaining the sediment.
2. Resuspend the sediment with 10 ml of 10% buffered formalin and mix the specimen well.
3. Add 5 ml of ethyl acetate or substitute. Cap the tube, invert the tube and shake vigorously for 30 seconds. Remove the cap with care, pointing the tube away from you, since pressure can build up within the tube during shaking.
4. Centrifuge at 500–600 xg for 10 minutes. Following centrifugation, four layers will develop:
   a. A top layer of mostly ethyl acetate
   b. An interface layer of fatty fecal debris
   c. A lower solution layer
   d. A pellet/sediment layer
5. Holding the centrifuge tube in a vertical position, free the plug of debris from the sides of the tube by ringing the tube with a wood applicator stick. Carefully pour the top three layers into an appropriate discard container. While the tube is still tipped in the decanting position, use a cotton-tipped swab to remove debris from the sides of the tube. Do not turn the tube upright until the sides of the tube have been thoroughly cleaned. If the sides are not cleaned thoroughly with the swab, lipid droplets can mix with the sediment making the examination much more difficult.
6. Prepare wet mount of the sediment on a clean glass microscope slide. Examination should be performed within 30 minutes. If mounts are to be prepared later, a small amount of 10% buffered formalin may be added to the sediment and the tube capped off. Consult appropriate references for the proper examination of the sediment and identification of parasites.

CALCULATIONS
N/A

RESULTS

Parasite	n=	**10% Formalin**		**SAF**		**PROTO-FIX**		Recovery Improvement with PARA-PRO *fc50*	
		PARA-PRO *fc50*	Para-Pak® Macro-CON®	PARA-PRO *fc50*	Para-Pak® Macro-CON®	PARA-PRO *fc50*	Para-Pak® Macro-CON®		
A. lumbricoides	15	3.7	1.5	1.5	0.9	5.5	2.7	+ 100%	p < 0.0001
B. hominis	3	27.4	18.8	15.8	12.6	36.5	22.9	+ 59%	p < 0.0001
C. mesnili	1	7	3	4	0	11	5	n.d.	n.d.
D. fragilis	3	0	0	0	0	4.6	2.3	n.d	n.d
E. coli	24	13.5	8.8	1.4	4.7	21.5	13	+ 66%	p < 0.0001
E. nana	11	8.6	5	3.5	1.8	12.3	0.4	+ 67%	p < 0.0001
E. histolytica	7	10.3	7.1	6.7	4.3	14.9	10	+ 49%	p < 0.0004
G. lamblia	7	19.9	15.4	12.9	9.4	26.8	19.1	+ 40%	p < 0.0012
Hookworm	29	2.9	1.3	1.1	0.5	3.9	1.6	+ 144%	p < 0.0001
I. butschlii	5	10.4	7.2	6	3.8	17.2	10.4	+ 65%	p < 0.0008
S. stercoralis	7	0	0	0	0	1.9	0.11	n.d.	n.d.
T. trichiura	6	3.5	1.8	1.6	1	5	2.3	+ 116%	p < 0.0030
Average parasites recovered per 50 microliter specimen n = number of positive samples									

The sensitivity and specificity of any O&P analysis is determined by the technique and experience of the product user; however, the PARA-PRO *fc50* was shown in a clinical study to improve sensitivity without affecting specificity. In an examination of 51 fecal specimens obtained during a Washington State University study, the efficiency of the PARA-PRO *fc50* was compared to the closest established device, the Para-Pak® Macro-CON® (Meridian Bioscience). Each specimen was divided into three portions, each preserved in different fixatives - 10% formalin, SAF and PROTO-FIX. Each fixed specimen was further divided and concentrated with each device after which ova and parasites were quantified by microscopic examination of 50 microliters of concentrate. The results of this study (see chart), show that the PARA-PRO *fc50* can improve the yield of a wide range of intestinal parasites over that achieved with a similar device. Regardless of the fixative used, dramatic and significant improvement in recovery was observed with the PARA-PRO *fc50* and in several samples this was a limiting factor in the observance of low-population parasites. For example, in six of seven positive samples, *S. stercoralis* was only observed with the combined use of PARA-PRO *fc50* and PROTO-FIX. The PARA-PRO *fc50* provides comparable results to the formalin-ethyl acetate concentration procedure described by Ritchie and as modified by Young, et al.

Notes:
1. If the fresh fecal sample is watery, the specimen should first be centrifuged for 3–5 minutes at 450–500 xg. Carefully decant the supernatant and use the sediment for concentration procedures.
2. The PARA-PRO *fc50* unit has a precision molded filtration screen, which allows helminth eggs and larvae, and protozoan cysts and trophozoites to pass but retains larger diameter particulate matter on the screen. Most of the macroscopic fecal debris will be discarded with the funnel/filtration unit. With dense fecal samples, the flow rate will be much slower. However, do not force the sample through the filtration device by any method. Such action could damage the filtration unit requiring the sample to be retested. Also, scraping or forcing the sample material through the funnel/filtration device may force material through the device which will result in non-standardized sized particulates in the final sediment. This larger size material will make examination more difficult and could make coverslipping difficult.

COMMENTS
PRECAUTIONS
1. **To be used by trained qualified laboratory personnel only.**
2. CAUTION! Ethyl acetate is FLAMMABLE. Perform all procedures in a well-ventilated area. Do not allow any open flames or ignition devices in the room when these procedures are being performed. Avoid prolonged breathing of fumes. Avoid skin contact or contact with eyes. Wear gloves and eye protection at all times while performing these procedures.
3. Observe standard Good Laboratory Practices in handling and disposing of bio-hazardous clinical specimens and laboratory reagents. Refer to your facility safety director for specific details.
4. Do not use the product if the expiration date on the reagents has been exceeded.

LIMITATIONS
N/A

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CONTACT
Alpha-Tec Systems, Inc. offers complete line of reagents, stains, QC Slides, O&P Collection Systems and Concentration Systems for Parasitology. For Technical Assistance email Technical@AlphaTecSystems.com, for Customer Service email Sales@AlphaTecSystems.com or call [+1] 800.221.6058 or [+1] 360.260.2779 between 8 am and 4 pm Monday through Friday, Pacific Time.

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TRADEMARKS:

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GLOSSARY OF SYMBOLS

LOT Batch code / Numéro de lot / Número de Lote / Numero di lotto / Lot Nummer / Lotnummer / Lotnummer / Šaržna številka / Número de lote

REF Catalog number / Référence du catalogue / Número de catálogo / Numero di catalogo / Katalognummer / Catalog nummer / Het aantal van de catalogus / Kataloška številka / Número de catálogo

IVD In vitro diagnostic medical device / Pour usage diagnostique in vitro / Para uso diagnóstico in vitro solamente / Solo per uso diagnostico in vitro / Nur zur Verwendung als in vitro-Diagnostikum / Alleen voor in vitro diagnostisch gebruik / För invitrodiagnostik enbart / Samo za invitro diagnostiko / Apenas para uso em diagnóstico in vitro

EC REP Authorized representative in the European Community / Représentant européen autorisé / Representante Europeo Autorizado / Rappresentante europeo autorizzato / Autorisierter Europäischer Repräsentant / Germachtigde Europese vertegenwoordiger / Auktoriserad europeisk representant / Pooblaščen evropski predstavnik / Representante Europeu Autorizado

Use-by date / Utiliser avant la date de péremption indiquée / Use antes de la fecha indicada / Utilizzare entro la data indicata / Bis zum angegebenen datum verbrauchen / Gebruik door vermelde datum / Använd innan angivet datum / Porabiti do navadenega datuma / Usar até à data indicada

Manufacturer / Fabricant / Fabricante / Produttore / Hersteller / Fabrikant / Fabrikant / Proizvajalec / Fabricante

Caution / Attention / Cuidado / Attenzione / Achtung / Voorzichtig / Iaktag försiktighet / Previdno / Atenção

Temperature limit / Conserver aux températures indiquées / Almacene entre las temperaturas indicadas / Conservare a temperature comprese fra quelle indicate / Im angegebenen temperaturbereich aufbewahren / Opslaan bij een temperatuur tussen / Förvara mellan angivna temperaturer / Shranjevati med navedenimi temperaturami / Armazene entre as temperaturas indicadas

Contains sufficient for <n> tests / Contenu suffisant pour <n> tests / Contiene suficiente para <n> pruebas / Contenuto sufficiente per <n> tests / Enthält ausreichend für <n> untersuchungen / Inhoud voldoende voor <n> testen / Innehåller tillräckligt för <n> tester / Vsebina zadostuje za <n> testov / Contém quantidade suficiente para <n> testes

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