PRINCIPLE
Intestinal parasitic infections are diagnosed by recovery and identification of protozoan trophozoites and / or cysts or helminth eggs and / or juvenile nematodes. Treatment is based upon positive identification of pathogenic species. As circumstances often prevent immediate examination of fecal specimens, the specimen must be preserved for later examination.

PROTO-FIX® fixative is designed for the collection, transport and preservation of fecal specimens for permanent staining and concentration procedures to diagnose intestinal parasites. PROTO-FIX fixative is a clear, colorless one vial processing fixative for wet preparations, permanent stains, concentrations, DFA, EIA and PCR methodologies for fecal specimens. PROTO-FIX is a low alcohol, low viscosity fixative that contains no heavy metals, PVA or aniline dyes.

PROTO-FIX fixative is an alternative to the traditional two-vial system of PVA fixatives and formalin. PROTO-FIX fixed smears can be stained using modified Wheatley's Trichrome Stain. Protozoan trophozoites, cysts, helminth eggs and juvenile nematodes can be concentrated using the CONSED™ concentration procedure or the FEA [formalin ethyl acetate] procedure. The CONSED Concentration Reagent in conjunction with the performance characteristics of PROTO-FIX yield equal or better concentration and staining results than the conventional methodologies (PVA and 10% formalin) and provides the convenience and cost savings of a single-vial system. PROTO-FIX fixative has no PVA, mercury, zinc or other heavy metals.

The CONSED concentration procedure concentrates all stages of all parasites present in the fecal specimen, including protozoan trophozoites. Trophozoites are clearly visible and identifiable on slides prepared from the concentrated specimen. PROTO-FIX, when used with CONSED will also concentrate higher numbers of juvenile nematodes [*Strongyloides* spp.]. PROTO-FIX fixative, unlike 10% formalin and SAF, kills fertilized eggs of *Ascaris* spp. ELISA procedures and DFA procedures can be performed directly from fecal specimens collected and fixed in PROTO-FIX.

SPECIMEN COLLECTION AND PREPARATION
1. The patient should be instructed not to take antacids, oily laxatives or antidiarrheal medication unless prescribed by a physician, prior to the collection of the sample(s). Radiological examinations utilizing contrast chemicals (i.e., bismuth or barium) should be avoided prior to the collection of the fecal specimen for parasitic analysis, as they will block the staining of the ova & parasites.
2. Multiple fecal specimens are recommended to increase the predictive value of parasite recovery.
3. Fecal specimens should be passed into a dry, clean container. Do not contaminate the feces with water or urine.
4. Add enough specimen to each vial to reach the "FILL LINE" found on the label of the vial. When present, slimy, watery or bloody portions should be collected.
5. Mix the specimens well. Cap and shake the vial vigorously.
6. Label the vials with appropriate patient information and return them to the laboratory. Do not freeze or incubate the specimen / fixative vials. Keep the specimen / fixative vials from heat.
7. The well-mixed specimen must be fixed for a minimum of 1 hour to assure adequate fixation of the parasitic elements.
   FOR IN VITRO DIAGNOSTIC USE ONLY.

REAGENTS AND MATERIALS
1. **Provided**
   a. PROTO-FIX may be packaged alone, in a single patient vial set or in a two-vial patient collection set (clean vial or ETM™).
   b. PROTO-FIX is available in various fill volumes.

2. **Not Provided**
   a. Pasteur pipettes
   b. Centrifuge with a free-swinging head
   c. Microscope/ Microscope slides and coverslips
   d. Cotton-tipped applicators
   e. Reagents and materials for staining and concentration

3. **Recommended additional reagents and materials**
   a. CELL-BOND™ microscope slides (#0003257)
   b. CONSED, ethyl acetate (#0003344)
   c. PRS™ (#0004044) or PARA-PRO® *FC50* (#0004060) Concentration System

4. **Storage:** Store at room temperature (15º-30º C). Avoid excessive heat and sunlight.

CALIBRATION
N/A

QUALITY CONTROL
PROTO-FIX fixative is a colorless, clear, precipitate-free liquid. If excessive precipitation occurs, discard the fixative vial. The fixative should not be used beyond the product expiration date. The use of a positive parasite control slide for permanent staining procedures is recommended. The PROTO-FIX Quality Control Slides (#0003255) contain *G. lamblia* trophozoites, ready for staining.

PROCEDURE

CONCENTRATION PROCEDURES
NOTE: There are two concentration procedures provided below. Only one concentration procedure needs to be performed. The laboratory may select the CONSED Concentration Procedure or the Formalin / Ethyl Acetate Concentration Procedure. It is highly recommended that the CONSED Concentration Procedure be selected.

CONSED CONCENTRATION PROCEDURE
NOTE: The CONSED Concentration Reagent is recommended as it increases the recovery of ova, helminths and parasites. In addition, the CONSED concentration procedure increases laboratory efficiencies and the diagnostic value of the permanent stain, as the permanent smear can be performed from the CONSED concentrated pellet.

1. Thoroughly mix the PROTO-FIX fixed specimen by shaking the specimen / fixative vial.
2. Add 4-5 drops of 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 10 to 20 seconds.
4. Place a PRS concentration funnel or a PARA-*PRO FC50* concentration unit (or other filtration device) into an appropriate polypropylene centrifuge tube. Remix the fixed specimen and pour the sample through the funnel into the receiver centrifuge tube. **NOTE:** Do not force the fixed specimen solution through the funnel system.
5. Following the filtration process, retain the receiver centrifuge tube and discard the filtration unit into an appropriate disposal container.
6. Transfer 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.
7. Place the capped centrifuge tubes into the centrifuge (with a free swinging head) and centrifuge for 10 minutes at 500 to 600xg. 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
8. 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.
9. Add 3-6 drops (or an amount equal to the volume of the pellet) of PROTO-FIX. Using an applicator stick, mix thoroughly. (**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”.)
10. Prepare the wet mount by placing 1 drop of the pellet prepared in step #9 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.

FORMALIN / ETHYL ACETATE CONCENTRATION PROCEDURE
1. Thoroughly mix the PROTO-FIX fixed specimen by shaking the specimen / fixative vial.
2. Add 4-5 drops of 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 10 to 20 seconds.
4. Place a PRS concentration funnel or a PARA-*PRO FC50* concentration unit (or other filtration device) into an appropriate polypropylene centrifuge tube. Remix the fixed specimen and pour the sample through the funnel into the receiver centrifuge tube. **NOTE:** Do not force the fixed specimen solution through the funnel system.
5. Following the filtration process, retain the receiver centrifuge tube and discard the filtration unit into an appropriate disposal container.
6. Transfer 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.
7. 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.
8. Place the capped centrifuge tubes into the centrifuge (with a free swinging head) and centrifuge for 10 minutes at 500 to 600xg. 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

9. 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

10. 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.
11. Add a few drops of PROTO-FIX to the pellet and mix well.
12. Prepare the wet mount by placing 1 drop of the sediment prepared in step #10 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.

PREPARING SLIDES FOR SMEARS

PROTO-FIX contains no PVA and as such requires the use of an adhesive to improve the adhesion of the specimen to the slide during routine staining procedures. One of the following methods should be used in the slide preparation:

1. The CELL-BOND Slides are ready to use bio-adhesive impregnated slides, which contain a charge, binding the specimen to the slide. Unlike Slide Coating Solution, Mayer's Albumin or PVA, CELL-BOND Slides provide no competitive staining background. This results in a clearer microscopic view of the fecal smear.

PERMANENT SMEAR PREPARATION:

1. Transfer 1-2 drops of the CONSED concentrated pellet or 1-2 drops of the unconcentrated PROTO-FIX fixed specimen to a CELL-BOND slide (see above).
2. Gently and evenly spread the sample over the microscope slide. Lay or hold the slide flat with the specimen side up. Using an applicator stick, chop the specimen, spreading specimen out. This will create thick and thin areas. Allow the slide to remain flat for 1 to 2 minutes. If there is still excessive liquid on the slide, stand the slide in a drying rack at 45 degrees to allow any excess liquid to drain. **NOTE:** Slides should dry for 10-15 minutes. Fecal films / smears dry at different rates. If some fecal smears are still wet after 15 minutes, they may be air-dried with a cool fan or blower (Do not use heat to dry the fecal smear slides). Fecal films / smears can be slightly wet when staining begins. When the excess liquid stops draining (usually about 5 to 10 minutes), carefully wipe away any excess liquid on the end or edges of the slide and place the slides into a carrier for staining.

PERMANENT STAIN PROCEDURE
Modified Wheatley's Trichrome Stain Procedure

Reagent	Timing
70% Ethanol (0003359)	1.5 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material.
70% Ethanol	1.5 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material.
Wheatley's Trichrome Stain (0003351)*	13 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material.
90% Acid-Ethanol (0003350)	5 seconds. Remove and drain off excess liquid by touching edge of slide to absorbent material.
100% Ethanol (0003303)	10 seconds. Remove and drain off excess liquid by touching edge of slide to absorbent material.
100% Ethanol	1 minute. Remove and drain off excess liquid by touching edge of slide to absorbent material.
100% Ethanol	1 minute. Remove and drain off excess liquid by touching edge of slide to absorbent material.
Xylene (0003342) or PRO-Clear™ (0003336) or ESC-1™ (0004401)	3 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material and coverslip with mounting fluid. Read under oil immersion.

NOTE: The xylene substitute AmeriClear® does not yield appropriate stain results and should not be used.

*** NOTE:** Staining time can be varied depending on the intensity desired for the final stain.

MISCELLANEOUS PROCEDURES

Cryptosporidium Staining Procedure

1. Place 1 drop of the specimen onto a clean glass slide. Using an applicator stick spread the specimen evenly to achieve a thin layer on the slide. Allow the slide to dry for 5 to 10 minutes before proceeding.
2. Dip the dried smear into 70% ethanol for 1 minute.
3. Remove the smear from the ethanol and gently shake off any excess ethanol. Dip the smear into distilled water about 8 times to sheet off the ethanol. Shake off the excess water and stain with the Cryptosporidium Stain Set (#0003357) or other appropriate stains.

DFA Staining Preparation

1. Follow the smear preparation procedure listed above under **"Cryptosporidium Staining Procedure"** to prepare a slide for DFA staining.
2. Follow the specific DFA manufacturer's directions for staining the smear for DFA *Cryptosporidium parvum* and *Giardia lamblia*.
3. PROTO-FIX has been validated against the MERIFLUOR® DFA stain for *Cryptosporidium parvum* and *Giardia lamblia*.

Microsporidium Staining Procedure

1. Follow the smear preparation procedure listed above under **"Cryptosporidium Staining Procedure"** to prepare a slide for Microsporidium staining.
2. Follow the specific Microsporidium Stain manufacturer's directions for staining the smear.

Enzyme Immunoassay (EIA) Procedure

The PROTO-FIX has been validated against the following manufacturers' products for EIA testing for *Cryptosporidium parvum* and *Giardia lamblia.*

1. ProSpecT™ *Cryptosporidium* Rapid Assay
2. ProSpecT™ *Cryptosporidium* Microplate Assay
3. Wampole CRYPTOSPORIDIUM TEST
4. *Xpect*® *Cryptosporidium* Kit
5. ProSpecT™ *Cryptosporidium parvum* and *Giardia lamblia.* Microplate ELISA Assay
6. ColorPAC™ *Giardia/Cryptosporidium* Rapid Test
7. ImmunoCard STAT!® *Cryptosporidium/Giardia*
8. *Xpect*® *Giardia and Cryptosporidium* Kit
9. ProSpecT™ *Giardia/Cryptosporidium* Microplate Assay
10. Wampole GIARDIA TEST
11. ProSpecT™ *Giardia* Rapid Assay
12. *Xpect*® *Giardia* Kit

CALCULATIONS

N/A

RESULTS

PROTO-FIX fixative, when used according to the procedures listed above should yield quality microscopic morphologies from the wet preparation, permanent stain concentration procedures. Using the modified Wheatley's Trichrome Stain procedure with organisms fixed in PROTO-FIX, the cytoplasm of trophozoites and cysts is blue-green tinged with purple. The nuclear chromatin, chromatid bodies, erythrocytes and bacteria stain purple to purplish-red. Background and artifacts stain green to purple. Helminth eggs and larvae stain red to purplish-red.

COMMENTS

1. **PRECAUTIONS**
   a. PROTO-FIX is poisonous. If ingested, give plenty of milk or water. Contact a physician or poison center immediately.
   b. Avoid contact with skin and eyes. If contacted, wash thoroughly with water. Contact a physician if irritation occurs.
   c. Observe all safety precautions for handling stool specimens.
2. **MACROSCOPIC EXAMINATION**
   a. The fecal specimen should be examined for consistency.
   b. Record any visible irregularities such as worms, proglottids, mucus and / or blood.
3. **DIRECT MICROSCOPIC EXAMINATION**
   a. The use of microscopic examination holds limited diagnostic value and should be limited to prescreening of specimens in the field when a centrifuge is not available. If the laboratory is going to perform the concentration procedure and / or the permanent stain, the direct microscopic examination can be eliminated.

LIMITATIONS

1. The proper fixation of intestinal ova and parasites yields quality microscopic morphology and staining. Specimens not properly fixed [specimens delayed before being fixed, improper ratios of specimen to fixative and improper mixing of the specimen into the fixative] may yield poor microscopic morphology making it difficult or impossible to properly identify the ova or parasite. False negative examinations may occur if too little specimen or if too much specimen is used in the concentration procedures.
2. The Modified Wheatley's Trichrome stain referred to in this Directions For Use is designed for use with PROTO-FIX only. All proficiency specimens received for staining (usually Zn-PVA smears) must be stained using the modified Trichrome stain designed for the zinc PVA fixative (#0003125).
3. The xylene substitute AmeriClear® does not yield appropriate stain results and should not be used.

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Manufactured by Alpha-Tec Systems Inc., 1311 SE Cardinal Court, Suite 170, Vancouver, WA 98683 USA.

EC REP MDSS GmbH
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GLOSSARY OF SYMBOLS

LOT Lot number / Número de porción / Número de lote / Numerodi lotto / Partienummer / Numéro de sort / Het Aantal van de partij

REF Catalog number / Número de catálogo / Número de catálogo / Numero di catalogo / Katalogzahl / Numéro de catalogue / Het aantal van de catalogus

IVD For in vitro diagnostic use only / Para el uso diagnóstico in vitro solamente / Para in vitro o uso diagnóstico somente / Solo per uso diagnostico in vitro / Für nur in vitrodiagnosegebrauch / Pour l'usage diagnostique in vitro seulement / Voor kenmerkend slechts gebruik in vitro

EC REP Authorized European representative / Representante europeo autorizado / Representante europeu autorizado / Rappresentante europeo autorizzato / Autorisierter europäischer Repräsentant / Represent ant européen autorisé / Gemachtigde Europese vertegenwoordiger

Caution, consult accompanying documents / Precaución, consulte los documentos de acompañamiento / Cuidado, consulte originais acompanhando / Attenzione. Consulti i documenti di accompagnamento / Vorsicht, beraten Begleitdokumente / Attention, consultez les documents d'accompagnement / Voorzichtigheid, raadpleeg begeleidende documenten

Store between temperatures indicated / Almacén entre las temperaturas indicadas / Loja entre as temperaturas indicadas / Deposito fra le temperature indicate / Speicher zwischen den Temperaturen angezeigt / Magasin entre les températures indiquées / Opslag tussen vermelde temperature

Consult instructions for use / Consulte las instrucciones para el uso / Consulte instruções para o uso / Consulti le istruzioni per uso / Beraten Sie Anwendungsvorschriften / Consultez les instructions pour l'usage / Raadpleeg instructies voor gebruik

Use by date indicated / Uso por la fecha indicada / Uso pela data indicada / Uso entro la data indicate / Gebrauch bis zum der Dattel angezeigt / Utilisation à la datte indiquée / Gebruik door vermelde datum

Manufacturer / Fabricante / Fabricante / Fornitore / Hersteller / Fabricant / Fabrikant

Contains sufficient for <n> tests / Contiene suficiente para <n> las pruebas / Contem suficiente para <n> testes / Contiene sufficiente per <n> le prove / Enthält genügendes für <n> tests / Contient suffisamment pour <n> des essays / Bevat voldoende voor <n> tests

