Para-Pak®/Para-Pak® ULTRA Systems
For use with SAF
(US Patent No. 5624554)

REF **300212, 300412, 330384, 380212,900212, 910212, 980212, 990160**

IVD

Rx Only

INTENDED USE
The Para-Pak/Para-Pak ULTRA SAF is a convenient system for the routine collection, transportation, preservation, and examination of stool specimens for intestinal parasites. It is designed for easy use by individuals not trained in microbiological techniques and affords an excellent means of minimizing the adverse effects of delay in specimen transportation.

SUMMARY AND EXPLANATION OF THE TEST
Diagnosis of intestinal parasitic disease is confirmed by recovery and identification of helminth eggs and larvae, or protozoan trophozoites and cysts in the clinical parasitology laboratory. Timely collection and transportation of "fresh" stool specimens to the laboratory cannot always be assured. Workload conditions and priorities in clinical laboratories frequently do not permit immediate examination of "fresh" specimens. Procedures such as incubation, refrigeration, or freezing will not guarantee the recovery of all diagnostic stages of all parasites.[1, 2-5, 8, 9]

In 1972, Junod described sodium acetate-acetic acid-formalin (SAF) fixative as a multipurpose fixative-preservative permitting the recovery and identification of all diagnostic stages of intestinal parasites. In a study of over 900 specimens Scholten and Yang confirmed the suitability of SAF fixative for routine use in the clinical parasitology lab as an alternative to other fixatives.[3, 10]

Proper use of the Para-Pak/Para-Pak ULTRA SAF systems thus assures the parasitologist that diagnostic stages of intestinal parasites will be preserved if present in the fecal material.

BIOLOGICAL PRINCIPLES
SAF provides a multipurpose fixative-preservative suitable for a variety of parasitological procedures. A direct examination may be made by diluting a drop of SAF preserved suspension in either saline or iodine. SAF preserved specimens also allow permanently stained mounts and a concentration procedure for direct examination.

The incorporation of an unpreserved specimen permits amoeba culture, rearing of hookworm larvae, bacterial culture, occult blood, and stool fat examination.[10]

REAGENTS/MATERIALS PROVIDED
The maximum number of tests obtained from this test kit is listed on the outer box.
1. SAF fixative: Each kit consists of one vial containing 15 mL SAF fixative with a built-in collection spoon. SAF fixative vials are also available in case quantities. Simple directions for patients and nursing personnel are also provided. Cases of Para-Pak ULTRA also contain two vials of surfactant.

MATERIALS NOT PROVIDED
1. Ethyl acetate (suggested) or dimethyl ether (optional)
2. Physiological saline
3. Cotton tipped applicator sticks
4. Microscope slides and coverslips
5. Centrifuge
6. Microscope
7. Transfer pipettes

PRECAUTIONS
1. All reagents are for in vitro diagnostic use only.
2. Ethyl acetate and dimethyl ether are flammable. Use in a well ventilated area. Avoid open flame. Avoid contact of the solution with skin and eyes. Should contact occur, flush with running water. Avoid breathing fumes.
3. Avoid contact of SAF solution with the skin and eyes. Should contact occur, flush with running water. If irritation develops contact a physician.
4. SAF solution is poisonous. If ingested, dilute by drinking milk or water. Call the local poison center or physician immediately.
5. Due to the infectious nature of unpreserved stools, use of gloves, care, and handwashing should be employed when the specimen is obtained and manipulated.

HAZARD and PRECAUTIONARY STATEMENTS

Para-Pak 10% Formalin and
Para-Pak ULTRA 10% Formalin

Signal Word
Danger
Hazard Statements
H301 - Toxic if swallowed
H311 - Toxic in contact with skin
H317 - May cause an allergic skin reaction
H341 - Suspected of causing genetic defects
H350 - May cause cancer
H402 - Harmful to aquatic life
H330 - Fatal if inhaled
H315 - Causes skin irritation
H318 - Causes serious eye damage
H370 - Causes damage to organs
Precautionary Statements - EU (§28, 1272/2008)
P301 + P310 - IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician
P321 - Specific treatment (see supplemental first aid instructions with this material)
P280 - Wear protective gloves/ protective clothing
P403 + P233 - Store in a well-ventilated place. Keep container tightly closed
P280 - Wear eye protection/ face protection
P321 - See SDS Section 4 or Section 11 for specific medical treatment information
P201 - Obtain special instructions before use
P281 - Use personal protective equipment as required
P308 + P313 - IF exposed or concerned: Get medical advice/ attention
P202 - Do not handle until all safety precautions have been read and understood
P264 - Wash face, hands and any exposed skin thoroughly after handling
P270 - Do not eat, drink or smoke when using this product
P260 - Do not breathe dust/fume/gas/mist/vapors/spray
P271 - Use only outdoors or in a well-ventilated area
P284 - Wear respiratory protection
P272 - Contaminated work clothing should not be allowed out of the workplace
P307 + P311 - IF exposed: Call a POISON CENTER or doctor/ physician
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P310 - Immediately call a POISON CENTER or doctor/ physician
P302 + P352 - IF ON SKIN: Wash with plenty of soap and water
P312 - Call a POISON CENTER or doctor/ physician if you feel unwell
P361 - Remove/Take off immediately all contaminated clothing
P332 + P313 - If skin irritation occurs: Get medical advice/ attention
P363 - Wash contaminated clothing before reuse
P304 + P340 - IF INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing
P310 - Immediately call a POISON CENTER or doctor/ physician
P405 - Store locked up
P403 + P233 - Store in a well-ventilated place. Keep container tightly closed
P501 - Dispose of contents/ container to an approved waste disposal plant.

Para-Pak SAF and
Para-Pak ULTRA SAF

Signal Word
Danger
Hazard Statements
H301 - Toxic if swallowed
H311 - Toxic in contact with skin
H318 - Causes serious eye damage
H341 - Suspected of causing genetic defects
H350 - May cause cancer
H315 - Causes skin irritation
H330 - Fatal if inhaled
Contains Formaldehyde
Precautionary Statements - EU (§28, 1272/2008)
P301 + P310 - IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician
P321 - Specific treatment (see supplemental first aid instructions with this material)
P321 - See SDS Section 4 or Section 11 for specific medical treatment information
P403 + P233 - Store in a well-ventilated place. Keep container tightly closed
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P310 - Immediately call a POISON CENTER or doctor/ physician
P281 - Use personal protective equipment as required
P201 - Obtain special instructions before use
P308 + P313 - IF exposed or concerned: Get medical advice/ attention
P304 + P340 - IF INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing
P310 - Immediately call a POISON CENTER or doctor/ physician
P302 + P352 - IF ON SKIN: Wash with plenty of soap and water
P310 - Immediately call a POISON CENTER or doctor/ physician
P362 - Take off contaminated clothing and wash before reuse
P308 + P313 - IF exposed or concerned: Get medical advice/ attention
P363 - Wash contaminated clothing before reuse
P333 + P313 - If skin irritation or rash occurs: Get medical advice/ attention
P330 - Rinse mouth
P405 - Store locked up
P403 + P233 - Store in a well-ventilated place. Keep container tightly closed
P501 - Dispose of contents/ container to an approved waste disposal plant.
P280 - Wear protective gloves/ protective clothing/ eye protection/ face protection
P202 - Do not handle until all safety precautions have been read and understood
P264 - Wash face, hands and any exposed skin thoroughly after handling
P270 - Do not eat, drink or smoke when using this product
P260 - Do not breathe dust/fume/gas/mist/vapors/spray
P271 - Use only outdoors or in a well-ventilated area
P284 - Wear respiratory protection
P272 - Contaminated work clothing should not be allowed out of the workplace

Reagent A (MucoPenX), CON-Trate Reagent A and
Macro-CON Surfactant

Signal word
Warning
Hazard Statements
H302 - Harmful if swallowed
Contains Polyethylene glycol 1,1,3,3-tetramethylbutylphenyl ether

SHELF LIFE AND STORAGE
Expiration dating for Para-Pak/Para-Pak ULTRA SAF is indicated on the outer packaging. Store at room temperature (15-30 C). Excessive heat and cold should be avoided.

SPECIMEN COLLECTION AND PREPARATION
1. The patient should be cautioned against the use of antacids, barium, bismuth, antidiarrheal medication, or oily laxatives prior to collection of the specimen.
2. To assure recovery of parasitic elements which are passed intermittently and in fluctuating numbers, three specimens spaced a few days apart must be examined. In the case of hospitalized patients it is suggested that all fecal passages be collected for a designated length of time to avoid prolonging the hospital stay.[1, 4]
3. The specimen is ideally passed into a bedpan but must not be contaminated with urine. Alternatively, a large plastic bag or "saran wrap" may be placed over the toilet seat opening and the specimen passed into the bag. A thoroughly cleaned and dried milk carton, cut to remove the upper two third of the carton, may also be used. It will be easier to collect the specimen if the water supply to the toilet is shut off and water drained from the bowl by "flushing twice".
4. An appropriate (i.e. bloody, mucoid, watery) area of stool should be selected and sampled with the collection spoons provided in the caps of the containers. Sufficient stool is added to each container to bring the liquid level up to the "Fill to Here" line. This will result in approximately 5 mL of sample. To insure adequate sampling of a formed stool, material should be removed from the sides, ends, and middle of the bolus.
5. Agitate each specimen with the spoon along the sides of the container, tighten the cap and shake firmly to insure that the specimen is adequately mixed. When mixing is completed the specimen should appear homogenous.
6. Return the vials to their container, seal the container, and label appropriately.

TEST PROCEDURE
The Para-Pak/Para-Pak ULTRA SAF Systems lend themselves to a wide variety of procedures in common use. The following discussion is not exhaustive and alternatives may be found in the literature cited. While variations may exist from lab to lab, a thorough examination should include at least three steps:
1. Gross examination: Record the presence of blood, worms, mucus, or proglottids.
2. Direct microscopic examination from the SAF preserved specimen:
   a. Place a clean glass slide on a sheet of newsprint.
   b. Add a drop of saline (iodine may be substituted if desired) to the slide.
   c. Add a representative sample of SAF preserved specimen to the drop of saline and mix thoroughly with the collecting spoon. The news print must be just legible through the slide.
   d. Place a double width coverslip on the suspension and examine immediately.
3. Permanent slide and concentration procedure using the Para-Pak ULTRA:
   a. **Permanent Slide Preparation:**
      1. Using the provided key, remove the protective cap from the vial.
      2. Snap and twist off the inner tip.
      3. If the specimen appears mucoid, add 10 drops of surfactant through the tip of the vial. Swirl gently to facilitate mixing.
      4. The vial can be used with a 15 mL or 50 mL conical tube. Insert the tube into the top of the Para-Pak ULTRA vial. Invert and filter (by squeezing the vial) at least 5 mL of specimen through the filtration device which is contained in the vial. (If desired, the entire specimen may be filtered.)
      5. While holding the vial and tube at a 30º angle, remove the vial from the conical centrifuge tube and add about 10 mL physiological saline to the tube and mix thoroughly.
      6. Centrifuge at 500 xg for 10 minutes (1800-2200 rpm for most tabletop centrifuges).
      7. Decant the supernatant fluid. Approximately 0.5 to 1.0 mL of sediment should remain. If necessary, remove sediment or add more filtered specimen and repeat steps 5-6.
      8. Mix the sediment and prepare a smear for permanent staining by following the steps 5a-f in Section #4 below.
   b. **Concentration procedure** (formalin-ether or ethyl acetate[11, 12] sedimentation):
      1. To the remaining sediment from Step a7 above, add 8 mL of 10% Formalin (or saline), mix and allow to stand for 5 minutes.[11]
      2. Add 3 mL of ethyl acetate, then stopper and shake the tube vigorously for at least 30 seconds. Carefully remove the stopper.
      3. Centrifuge for 10 minutes at 500-1000 xg (1800-2200 rpm for most tabletop centrifuges).
      4. Four layers will be apparent: (a) Top layer: ethyl acetate or ether, (b) Second layer: plug of debris, (c) Third layer: formalin, (d) Bottom layer: sediment.
      5. After ringing the plug of debris from the sides of the tube with an applicator stick, carefully decant the top three layers. While keeping the tube inverted, a cotton swab may be used to remove debris sticking to the sides of the tube. This is particularly important for obtaining suitable results with ethyl acetate and avoids solvent bubbles in the wet mount.
      6. Add a few drops of physiological saline or 10% Formalin to re-suspend the remaining sediment. If the resulting slides are too dense (news print should be legible through them) more saline or formalin may be added.
      7. Iodine and saline mounts are suggested for microscopic examination.

      NOTE: If the pellet in step 6 contains a large amount of debris, a wash step may be performed. Re-float the sediment in 7 mL water, shake, and re-centrifuge at 500 xg. Pour off supernatant and continue with steps 6 and 7.

4. Permanent slide and concentration procedure using the Para-Pak® Macro-Con® or Con-Trate® Stool Concentration System:
   a. Thoroughly mix the material in the Para-Pak SAF specimen collection vial until it appears homogeneous. The specimen is now ready for processing with the Para-Pak Macro-Con stool concentration system. Follow the Para-Pak Macro-Con package insert, Specimen Processing, steps 1 through 8. Insert the following procedure between steps 8 and 9 of the Macro-Con Specimen Processing section. (For Con-Trate, refer to product package insert.)
      1. Add physiological saline to bring the level of the filtered specimen to the dotted line on the Para-Pak Macro-Con label.
      2. Place a provided screw cap on the conical centrifuge tube and centrifuge for 10 minutes at 500-1000 xg (1800-2200 rpm for most tabletop centrifuges).
      3. Carefully pour off the supernatant fluid.
      4. Mix the sediment with an applicator stick in the saline which drains back from the walls of the tube. A small drop of saline may be added if necessary.
      5. Prepare a smear for permanent staining as follows (6, 7):
         a. Add a small drop of Mayer's albumin (supplied with each pack of 10 kits) to a clean glass slide and wipe immediately so that only a thin coating remains on the slide. NOTE: If slides have a reddish tinge after decolorization, too much albumin has remained on the slide.
         b. Add a small amount of the suspended sediment to the albumin coated slide.
         c. Spread the mixture over the slide to produce a film which varies in thickness.
         d. Allow the slide to dry 5-10 minutes at room temperature until "tacky".
         e. Immerse the slide in 70% ethanol for approximately 30 minutes to coagulate the albumin.
         f. Proceed with the staining regimen of choice. Wheatley's Trichrome (Meridian Catalog No. 400101) works particularly well with this method. Omit steps 1-3 in the staining section of the Wheatley's Trichrome package insert.
   b. Use the remaining sediment to follow Specimen Processing steps 9-18 in the Para-Pak Macro-Con package insert. If the Para-Pak Macro-Con is unavailable the following procedure for permanent slides and concentration may be implemented:
      1. Permanent slide preparation:
         a. Thoroughly mix the material in the SAF tube until it appears homogenous.
         b. Strain approximately 2-3 mL (amount will vary in direct proportion to the density of the suspension) of the suspended material through one layer of narrow mesh or two layers of wide mesh gauze into a suitable centrifuge tube.
         c. Add about 10 mL of physiological saline to the tube and mix thoroughly.
         d. Centrifuge 10 minutes at 500 xg.
         e. Decant the supernatant fluid. Approximately 0.5 to 1.0 mL of sediment should remain. If necessary, remove sediment or add more strained suspension and repeat steps c and d.
         f. Mix the sediment and prepare the permanent smear as described above, step 5a-f.
      2. Use the remaining sediment in the concentration procedure and follow step 3b listed above.

      NOTE: The zinc sulfate flotation procedure is reported to give poor results with this fixative and is not recommended. The flotation procedure may, however, be applied to the unpreserved specimen provided it is relatively fresh.[7]

QUALITY CONTROL

This test should be performed per applicable local, state, or federal regulations or accrediting agencies.

The Para-Pak/Para-Pak ULTRA SAF vial should contain approximately 15 mL of fluid to insure 1:3 stool to preservative ratio. When SAF fixed film of stock trophozoite or human buffy coat is stained the organisms or cells should appear well fixed and defined.

If the expected control reactions are not observed, repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor.