Para-Pak® PLUS EcoFix®

**ENGLISH**

**REF** Cat. No. **950620** (200 TESTS) – **950605** (50 TESTS)  

**IVD in vitro diagnostic use**

**INTENDED USE**

Para-Pak® PLUS EcoFix® is a convenient one-vial system for the routine collection, transportation, preservation and filtration of stool specimens for intestinal parasites. Both concentration and permanent stain may be performed from the EcoFix® preserved specimen. The fixative affords an excellent means of minimizing the adverse effects of delay in specimen transport.

**EXPLANATION**

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, 3, 4, 5, 8, 9).

Proper use of Para-Pak® PLUS EcoFix® thus assures the parasitologist that diagnostic stages of intestinal parasites will be preserved. EcoFix® was developed as a formalin-free and mercury-free fixative in response to disposal problems and monitoring regulations (10).

**CHEMICAL AND PHYSICAL PRINCIPLES**

EcoFix® provides a multi-purpose mercury and formalin-free fixative/preservative suitable for a variety of parasitological procedures. EcoFix® preserved specimens may be concentrated and used to prepare permanently stained slides.

**KIT COMPONENTS**

- Vials containing 13 ml of EcoFix® fixative and a built-in collection spoon for sample collection.
- Surfactant
- Wooden spatulas
- Full instructions for professional use
- Simple directions for sample collection for patients and nursing personnel

**MATERIALS NEEDED BUT NOT PROVIDED**

- Lugol’s Iodine
- Microscope Slides and Cover Slips
- Microscope

To perform standard concentration procedure are also needed:

- Centrifuge Tubes (15 ml)
- Physiological Saline Solution (or 10% Formalin)
- Ethyl Acetate (REF. FK4318) or Hemo-De (REF. 801300)
- Cotton Tipped Applicator Sticks
- Transfer Pipettes
- Centrifuge

**STABILITY AND STORAGE**

Store at room temperature in upright position, in a well ventilated place. Avoid exposure to heat or direct light.

Shelf life of the Para-Pak® PLUS EcoFix® is two years when stored at room temperature. Refer to expiry dates reported on the labels.
PROTOCOL

The Para-Pak® PLUS EcoFix® System lends itself to a wide variety of procedures in common use. The following discussion is not exhaustive and alternatives may be found.

PRECAUTIONS related to SAMPLE COLLECTION

1. Stool specimens should be fresh and untreated. Fresh stool should be fixed within 30 minutes from collection.
2. The patients should be cautioned against the use of antacids, barium, bismuth, antidiarrheal medication, or oily laxatives prior to collection of the specimen.
3. To assure recovery of parasitic elements that are passed intermittently and in fluctuating numbers, three specimens spaced 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.
4. The specimen must not be contaminated with urine.
5. Provide patients with proper instructions.

STEP 1 - SPECIMEN COLLECTION

1. The specimen is ideally passed into a bedpan but must not be contaminated with urine. Alternatively, a large plastic bag or wrapping film may be placed in the toilet opening and the specimen passed into the bag.
2. An appropriate (i.e. bloody, slimy, watery) area of stool should be selected and sampled. Use the spatula to fill the sample collector attached to the vial cap. One scoopful is added to each container. This will result in approximately 1 ml (or 1 gram) of sample. Do not add more than the collector cavity can hold. Liquid specimens should be collected using a small spoon. The sample amount should just fill the concave side of the homogenizer. (The white plastic part attached at the transport vial cap). Excessive or low amount of sample, could deliver worse results.
3. Tighten the cap and shake firmly to insure that the specimen is adequately mixed. When mixing is completed the specimen should appear homogenous.
4. Label appropriately and send the vials to the laboratory.

STEP 2 - SPECIMEN PROCESSING and ANALYSIS

1. If the specimen appears to be mucoid, add 5-10 drops of surfactant through the tip of the vial.
2. Mix the EcoFix® fixed specimen thoroughly.
Collected sample can be now analyzed directly (refer to section DIRECT SPECIMEN ANALYSIS) or concentrated before analysis (refer directly to section SPECIMEN CONCENTRATION).

DIRECT SPECIMEN ANALYSIS

A. Gross examination:
Analyze stool suspension and record the presence of blood, worms, mucus, or proglottids.

B. Direct microscopic examination (FRESH MOUNT)
1. Add a drop of saline to a microscope slide. (NOTE: if utilizing the direct exam, iodine cannot be used to stain the preparation).
2. Remove the cap from the tip, invert and filter some drops of sample by gently squeezing the vial on the drop of saline and mix thoroughly with an applicator stick. The newsprint must be just legible through the slide.
4. Place a double width coverslip on the suspension and examine immediately.

C. Permanent staining:
1. Prepare a permanent smear: remove the cap from the tip, invert and filter some drops of sample by gently squeezing the vial on the slide. The newsprint must be just legible through the slide.
2. Dry the slide overnight.
SPECIMEN CONCENTRATION and ANALYSIS

A. CONCENTRATION with ETHYL ACETATE/SALINE
1. Mix the EcoFix® fixed specimen thoroughly. Remove the green cap from the tip, invert and filter by gently squeezing the vial and placing the spout into a 15 ml centrifuge tube. Filter at least 5 ml of specimen through the filtration device contained in the vial. (If desired, the entire specimen may be filtered)
2. Centrifuge at 500 x g for 10 minutes (1800-2200 rpm for most table top centrifuges).
3. Decant the supernatant fluid. Approximately 0.5 to 1.0ml of sediment should remain. If necessary add more filtered specimen and repeat step 3 and 4.
NOTE: If the pellet in step 3 contains a large amount of debris, a wash step may be performed. Re-float the sediment in 7 ml saline, shake, and re-centrifuge at 500 x g. Pour off supernatant and continue with step 4.
4. Add to the sediment 9 ml of physiological saline solution (or 10% formalin), mix and allow to stand for 10 minutes (13).
5. Add 3ml of ethyl acetate (or Hemo-De), then stopper and shake the tube vigorously for at least 30 seconds. Carefully remove the stopper.
6. Centrifuge for 10 minutes at 500-1000 x g (1800-2200 rpm for most table top centrifuges). Four layers will be apparent:
   a. Top layer: ethyl acetate (or Hemo-De)
   b. Second layer: plug of debris
   c. Third layer: saline solution (or formalin)
   d. Bottom layer: sediment containing parasites
7. 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 should be used to remove excess ethyl acetate (or Hemo-De) and debris sticking to the sides of the tube. This is particularly important for obtaining suitable results with ethyl acetate (or Hemo-De) and avoids solvent bubbles in the wet mount.

B. SLIDE PREPARATION

FRESH MOUNT
1. Add a few drops of physiological saline (or 10% formalin) to re-suspend the sediment.
NOTE: Recommend re-suspension in 10% formalin if any delay in reading the concentrate is anticipated. If the resulting slides are too dense (newsprint should be legible through them) more saline or 10% formalin may be added. Do not use EcoFix® to resuspend the pellet if you want to perform iodine staining. Direct Iodine or saline mounts are suggested for microscopic examination.
2. Use a disposable pipette to mix the parasite concentrate thoroughly. Add 1 drop of the re-suspended sediment per slide.
3. Eventually stain the sediment adding a few drop of Iodine Solution (Lugol).
4. Place a double width coverslip on the suspension and examine by a light microscope following standard procedures.

PERMANENT STAINING
1. Resuspend the remaining sediment with a few drop of EcoFix®.
2. Prepare a smear and dry overnight.

USER QUALITY CONTROL
1. The Para-Pak® PLUS EcoFix® vial should contain approximately 13ml of fluid to ensure 1:3 stool to preservation ratio.
2. When an EcoFix® fixed film of stock trophozoites or human buffy coat is stained, the organisms or cells should appear well fixed and defined.
GENERAL PRECAUTIONS

1. For professional use only
2. Due to the infectious nature of unpreserved stools, gloves, care and hand washing must be employed when the specimen is collected and manipulated.
3. Patient specimens may contain HIV or other infectious agents and should be handled by properly trained personnel and disposed of as potential biohazards.
4. Avoid contact of fixative solution with the skin and eyes. Should contact occur, flush with running water. If irritation should develop, see a physician.
5. Fixative solutions are poisonous. If ingested, call local poison center or physician immediately.
6. Dispose specimen and reactive following local laws
7. Do not use the kit after the expiry date reported on the package.

SAFETY INFORMATION

PRODUCT: EcoFix®

Indication of danger

Xi - Irritant.
F - Highly flammable.
N - Dangerous for the environment.

RISK PHRASES

R11 - Highly flammable. R36 –Irritating to eyes. R51/53 - Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

SAFETY PHRASES

S60 - This material and its container must be disposed of as hazardous waste. S61 Avoid release to the environment. Refer to special instructions/safety data sheets. S9 - Keep container in a well-ventilated place. S24/25 - Avoid contact with skin and eyes. S45 - In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

BIBLIOGRAPHY