Para-Pak® CON-Trate®
Stool Concentration Kit System

REF **960050, 960200, 960500** IVD **Rx Only**

INTENDED USE
The Meridian Para-Pak CON-Trate System is a complete system for concentrating and recovering helminth eggs, larvae and protozoan cysts from feces.

SUMMARY AND EXPLANATION OF THE TEST
Correct diagnosis of intestinal parasitic infection depends on proper collection, transport, detection, and identification of parasites in fecal specimens.[1, 2, 4, 5, 7, 9]

The detection and identification of small numbers of helminth eggs and protozoan cysts from large volumes of feces is of critical importance to the diagnosis of intestinal parasitic infection.

Historically, fecal specimens fixed in 10% formalin have been used in concentration procedures for detection and identification of parasitic elements.[1, 2, 6, 9, 10, 12, 13] Investigators have reported on the use of other fixatives such as SAF.[12] The Meridian Para-Pak CON-Trate system combines proven methodology with recent advances that optimize detection and identification, and minimizes specimen preparation in a safe, standardized, easily performed manner.[8]

BIOLOGICAL PRINCIPLES
The Meridian Para-Pak CON-Trate System uses efficient, cost effective methods for recovering protozoan cysts, helminth eggs (including operculated eggs) and larvae, from preserved fecal specimens.
1. The addition of CON-Trate Reagent A and thorough mixing of the preserved specimen enhances the breakdown of fecal aggregates and mucus, thus freeing parasites.[8]
2. Filtering the stool-Reagent A suspension through the unique CON-Trate filtering device removes macroscopic fecal aggregates and debris.[8]
3. Shaking the stool-Reagent A-Reagent B Suspension forms a colloidal mixture. Centrifugation concentrates parasitic elements in a sediment layer. Most of the fecal debris is discarded with the supernatant fluid.
4. Substitution of CON-Trate Reagent B for ethyl ether in the system results in equivalent or better recovery of parasites.[6, 11, 13] CON-Trate Reagent B is less flammable than ethyl ether. No distortion or destruction of parasitic elements has been reported.[6, 11, 13]

REAGENTS/MATERIALS PROVIDED
The maximum number of tests obtained from this test kit is listed on the outer box.
1. CON-Trate filtering devices
2. CON-Trate disposable centrifuge tubes with caps
3. CON-Trate Reagent A (MucoPenX) dropper bottle
4. CON-Trate Reagent B (Ethyle Acetate)

MATERIALS NOT PROVIDED
1. Cotton-tipped applicator sticks
2. Microscope slides and coverslips
3. Physiological saline, 10% buffered formalin
4. Centrifuge
5. Microscope
6. Pipettes

PRECAUTIONS
1. All reagents are for in vitro diagnostic use only.
2. CAUTION: CON-Trate Reagent B is flammable. Perform all procedures in a well ventilated area. Avoid open flames and ignition devices. Avoid contact with the skin or eyes. Avoid breathing fumes.
3. Observe good technique in handling and disposal of all biohazardous clinical and laboratory specimens and material.
4. Concentration of fecal specimens for parasitic examination is only an integral part of the overall scheme for identification of intestinal parasites. Additional tests and procedures may be found in appropriate references.
5. This product must not be used if:
   a. The expiration date on the label has passed.
   b. Proper storage conditions have not been observed.

HAZARD AND PRECAUTIONARY STATEMENTS

FOR PRODUCTS 960050 and 960200  Ethyl Acetate / Reagent B	**Signal Word** Danger **Hazard Statements** H319 - Causes serious eye irritation H336 - May cause drowsiness or dizziness H225 - Highly flammable liquid and vapor EUH066 - Repeated exposure may cause skin dryness or cracking **Precautionary Statements - EU (§28, 1272/2008)** P370 + P378 - In case of fire: Use dry sand, dry chemical or alcohol-resistant foam for extinction P210 - Keep away from heat/sparks/open flames/hot surfaces. - No smoking
 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
Shelf life of the Para-Pak CON-Trate system is indicated on the outer package label. Store at 15-30 C. Do not freeze.

SPECIMEN COLLECTIONAND PREPARATION

A. **SPECIMEN COLLECTION**

1. The patient must be properly instructed and assisted in the collection of the fecal sample. Refer to Meridian Para-Pak collection/transport kit product inserts or appropriate references far recommended collection and transport methods.
2. Any specimen preserved in 10% formalin, SAF, or an unpreserved fecal sample may be used with the CON-Trate System.

B. **SPECIMEN QUALITY ASSURANCE**

To assure a suitable clinical specimen using the CON-Trate System, observe the following:

1. The specimen/preservative mixture must be stored for a minimum of 30 minutes after collection for adequate fixation. IMPORTANT: Mix contents thoroughly.
2. The specimen must have been maintained at room temperature.
3. Adequate sample must be present. (2-3) gm of stool in 15 mL fixative is recommended.

C. **SPECIMEN PROCESSING**

1. **Unpreserved Specimens** (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.[1, 2, 7, 12]
   a. Transfer 3-5 grams of unpreserved stool into 15 m L preservative of choice, for economy and convenience, use Meridian Para -Pak· 10% Buffered Neutral Formalin (Catalog #900412) or SAF (Catalog #900212). Mix stool/preservative mixture thoroughly, break up any lumps or fecal masses (shaking for one minute is usually sufficient). The stool/preservative mixture should stand for a minimum of 30 minutes for adequate fixation. Follow procedure far use below for the preservative selected.
2. **Immediate processing of unpreserved specimens**[9]
   a. Transfer 5-6 grams of unpreserved stool into 10-15 mL of physiological saline. Mix stool/saline mixture thoroughly, break up any lumps or fecal masses.
   b. Add 4 drops of CON-Trate Reagent A to the mixture. (Up to 8 drops of Reagent A may be added if the specimen is highly mucoid.)
   c. Cap and mix the contents thoroughly by shaking.
   d. Insert one of the CON-Trate filtering devices into the top of one of the disposable centrifuge tubes provided.
   e. Pour fecal suspension through the filtering device into the centrifuge tube. Usually 3 mL in the centrifuge tube is sufficient unless the fecal suspension is thin.
   f. Discard filtering device, add 10 mL physiological saline and centrifuge at 500 xg for 10 minutes (1800-2200 rpm). Decant the supernatant fluids, retaining the sediment. About 1 mL of sediment should be present. A portion of the sediment may be used for d etection of *Cryptosporidium.* Consult an appropriate reference for proper preparation and examination. (See Hint 6)
   g. Resuspend the sediment in 10 mL buffered formalin. Allow mixture to stand for at least 5 minutes before proceeding. (At this point, the mixture in the centrifuge tube may be capped and saved until a later time.)
   h. Add approximately 3 mL of CON-Trate Reagent B, cap the tube and shake for 30 seconds. Invert the tube while shaking. **CAUTION:** Pressure may build up within the tube during shaking. Carefully release the pressure by opening the cap on the centrifuge tube away from your person.
   i. Centrifuge the tube at 500 xg for 10 minutes (1800-2200 rpm). Examination of the tube after centrifugation should reveal four distinct layers from the top down:
      1. a layer consisting of Reagent B
      2. a "plug" of fecal debris
      3. a discolored aqueous layer
      4. a sediment layer, containing the parasites.

      The final sediment remaining should be approximately 0.25 mL.
   j. Hold the tube in a vertical position. Free the plug of debris by ringing with a wooden applicator stick. Decant the upper layers, leaving the sediment. **DO NOT TURN THE TUBE UPRIGHT UNTIL THE SIDES OF THE TUBE HAVE BEEN CLEANED WITH COTTON TIPPED SWABS.**
   k. Transfer a portion of the sediment to a clean glass microscope slide and prepare the mount of choice. Examine microscopically. Consult an appropriate reference for proper preparation and examination.[5, 7, 9]
3. **Formalin preserved specimens**
   a. Add 4 drops of CON-Trate Reagent A to the specimen in the fixative vial. (Up to 8 drops of Reagent A may be added if the specimen is highly mucoid)
   b. Cap and mix the contents thoroughly shaking.
   c. Insert one of the CON-Trate Filtering devices into the top of one of the disposable centrifuge tubes provided.
   d. Pour fecal suspension through the filtering device into the centrifuge tube. Usually 3 mL in the centrifuge tube is sufficient unless the fecal suspension is thin.
   e. Discard filtering device, add 10 mL saline and centrifuge at 500 xg for 10 minutes (1800-2200 rpm). Decant the supernatant fluids, retaining the sediment. A portion of the sediment may be used for detection of *Cryptosporidium.* Consult an appropriate reference for proper preparation and examination. (See Hint 6)
   f. Resuspend the sediment in 9 mL of 10% formalin.
   g. Add approximately 3 mL of CON-Trate Reagent B, cap the tube and shake for 30 seconds. Invert the tube while shaking. **CAUTION:** Pressure may build up within the tube during shaking. Carefully release the pressure by opening the cap on the centrifuge tube away from your person.
   h. Centrifuge the tube at 500 xg for 10 minutes (1800-2200 rpm). Examination of the tube after centrifugation should reveal four distinct layers from the top down:
      1. a layer consisting of Reagent B
      2. a "plug" of fecal debris
      3. a discolored aqueous layer
      4. a sediment layer, containing the parasites.

      The final sediment remaining should be approximately 0.25 mL.
   i. Hold the tube in a vertical position. Free the plug of debris by ringing with a wooden applicator stick. Decant the upper layers, leaving the sediment. **DO NOT TURN THE TUBE UPRIGHT UNTIL THE SIDES OF THE TUBE HAVE BEEN GLEANED WITH COTTON TIPPED SWABS.**
   j. Transfer a portion of the sediment to a clean glass microscope slide and prepare the mount of choice. Examine microscopically. Consult an appropriate reference for proper preparation and examination.[5, 7, 9]

4. **SAF preserved specimens**
   a. Add 4 drops of CON-Trate Reagent A to the specimen in the fixative vial (Up to 8 drops of Reagent A may be added if the specimen is highly mucoid).
   b. Cap and mix the contents thoroughly by shaking the vial several times.
   c. Insert one of the CON-Trate filtering devices into the top of one of the disposable centrifuge tubes provided.
   d. Pour fecal suspension through the filtering device into the centrifuge tube. Usually 3 mL in the centrifuge tube is sufficient unless the fecal suspension is thin.
   e. Discard filtering device, add 10 mL saline and centrifuge at 500 xg for 10 minutes (1800-2200 rpm). Decant the supernatant fluids retaining the sediment. A portion of the sediment may be used for detection of *Cryptosporidium* species. Consult an appropriate reference for proper preparation and examination. (See Hint 6)
   f. If permanent stained smears are to be prepared:
      1. Transfer some of the sediment to a drop of Mayer's Albumin on a slide.
      2. Mix well, then spread mixture over a clean, glass microscope slide producing an uneven film (thick and thin areas). This smear should be allowed to dry and can then be stained with a permanent stain such as Iron Hematoxylin or Wheatley's Trichrome (Catalog #400101).[2]
   g. Resuspend the sediment in 9 mL of 10% formalin.
   h. Add approximately 3 mL of Reagent B, cap the tube and shake for 30 seconds. Invert the tube while shaking. **CAUTION:** Pressure may build up within the tube during shaking. Carefully release the pressure by opening the cap on the centrifuge tube away from your person.
   i. Centrifuge the tube at 500 xg for 10 minutes (1800-2200 rpm). Examination of the tube after centrifugation should reveal four distinct layers from the top down:
      1. a layer consisting of Reagent B
      2. a "plug" of fecal debris
      3. a discolored aqueous layer
      4. a sediment layer, containing the parasites. The final sediment remaining should be approximately 0.25 mL.
   j. Hold the tube in a vertical position. Free the plug of debris by ringing with a wooden applicator stick. Decant the upper layers, leaving the sediment. **DO NOT TURN THE TUBE UPRIGHT UNTIL THE SIDES OF THE TUBE HAVE BEEN CLEANED WITH COTTON TIPPED SWABS.**
   k. Transfer a portion of the sediment to a clean glass microscope slide and prepare the mount of choice. Examine microscopically. Consult an appropriate reference for proper preparation and examination.[5, 7, 9]

SPECIFIC PERFORMANCE CHARACTERISTICS

The Meridian Para-Pak CON-Trate System, when used as directed under procedure for use, during clinical evaluation was found to provide results comparable/equivalent/superior[8] to the standard Ritchie-Formalin ether (Ethyl Acetate) sedimentation concentration procedure.

MucoPenX (Reagent A) has been formulated to break down the mucus present in stool specimens and will not interfere with staining procedures or cause distortion of parasites.

HINTS

Experience will dictate appropriate techniques and volumes to assure an adequate sediment for microscopic examination. The following is a list of hints and suggestions which will accomplish this objective.

1. **Volume of preserved fecal suspension to add through the filler device:** After the first centrifugation, a sediment of 1.0 mL is optimum. In a dense fecal suspension, with a ratio of stool to preservative of 1:3-1:5, 3.0 mL of filtrate will provide the optimum sediment volume. In less dense fecal suspensions, such as one would see in a watery stool, larger volumes of filtrate are necessary. In some instances, as much as 10.0 - 12.0 mL of filtrate may be necessary. The hint is that the **decreasing** density of the preserved, fecal suspension dictates a proportional **increase** in the filtrate volume. If sufficient fecal material was present in the original fecal suspension and care was taken in adjusting the filtrate volume, the final sediment volume, after concentration, optimally, should be 0.25 mL. Attention to detail and experience will provide the anticipated results.
2. **Fecal suspension should not be forced through the filter device:** With very dense fecal suspensions, the flow rate through the filter device is slow. Do not force the material through the fitter device by scraping with applicator sticks or washing the aqueous solutions. Such action often forces hard, grit-like material through the filter device. The presence of these materials in wet mount preparations results in a non-uniform flow or distribution of materials under the coverslip, making coverslipping difficult. The problem is minimized by adding the recommended amount of Reagent A and thoroughly mixing the specimen/fixative mixture. Vegetative matter may cover the screen in the filtration device; this may be removed by gently running an applicator stick through the material, or by tapping the side of the filtration device.
3. **Swabbing centrifuge tube:** Failure to swab the sides of the centrifuge tube after decanting the Reagent B, fecal debris plug and aqueous layer can result in a poor wet mount preparation. Allowing Reagent B, which is not miscible in the aqueous solutions, to run back into the sediment, will result in wet mount preparations which are not uniform due to the presence of the Reagent B.
4. **Sediment:** When the CON-Trate procedure is followed correctly, the sediment will appear dry and gritty. To facilitate reading, a drop of saline should be added to the sediment on the slide and the coverslip floated on top.
5. **Wet Preparation Theory:** Tradition has dictated that a direct microscopic examination be performed on stool specimens submitted to the laboratory. Tradition has perhaps obscured the rationale for this requisite. The direct microscopic examination of fresh, unpreserved, stool specimens or bowel materials facilitated the identification of protozoa by noting the characteristic motility of protozoan trophozoites. The common practice of submitting preserved stool specimens to the laboratory may eliminate the need for the direct microscopic examination. The detection rate of parasites may be increased by performing the first microscopic examination on the first centrifuged sediment, or by staining the sediment from an SAF preserved specimen with Wheatley's Trichrome or Iron Hematoxylin.
6. ***Cryptosporidium* Prep Using Para-Pak:**

   For loose or watery stools:

   After filtration of specimen, centrifuge sample at 500 xg for a full 10 minutes (1800-2200 rpm for most tabletop centrifuges).
   Decant the supernatant fluid. Approximately 0.5 to 1.0 mL of sediment should remain.
   Mix the sediment and prepare a smear for *Cryptosporidium.*

   For semi-solid or solid stools:

   Perform entire concentration procedure and prepare a slide from the sediment per lab protocol.