AlphaTec™
(800) 221-6058 Nationwide
(360) 260-2779 Washington

Directions For Use for the following:
CONSED™
Concentration Reagent

PRINCIPLE
Many methods have been developed for the concentration of cysts and eggs of parasites found in feces: brine flotation, zinc sulfate flotation, and formalin/ether sedimentation. Price modified the formalin/ether procedure for sedimentation of MIF-fixed fecal specimens. Because of the danger of using ether in the laboratory environment, ethyl acetate was substituted for ether.

The CONSED Concentration Reagent was developed for concentrating the parasites and eggs in fecal specimens by sedimentation. This system was designed especially for use on specimens fixed in Formalin, SAF, and PROTO-FIX®.

In a series of studies, the effectiveness of four different fixatives (10% Formalin, SAF, MIF, and PROTO-FIX) were tested, comparing the formalin-ethyl acetate procedure to the CONSED sedimentation procedure. Juvenile nematodes did not concentrate well using 10% Formalin and SAF, and the procedures using formalin-ethyl acetate with Formalin and SAF unsatisfactorily concentrated protozoan trophozoites (no trophozoites were found). On average, when the CONSED concentration procedure was used, the recovery rate of all stages and all forms of eggs and parasites was higher than the formalin-ethyl acetate method.

SPECIMEN COLLECTION AND PREPARATION
1. Specimens preserved in 5% or 10% Buffered Formalin, SAF, MIF Fixative, PROTO-FIX or fresh samples may be processed with CONSED. 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. An appropriate clinical patient sample, collected, preserved/fixed, and transported properly is important for the recovery of helminth eggs and larvae (juvenile nematodes) and protozoan trophozoites and cysts. Refer to the Directions For Use supplied with O&P Collection/Transport Sets for collection and transportation methods.
3. Always mix the sample well.
4. The appropriate volume of sample is 2 to 3 grams of fecal matter in 13 to 15 ml of fixative.
   FOR IN VITRO DIGNOSTIC USE ONLY

REAGENTS AND MATERIALS
1. **Provided**
   a. CONSED Concentration Reagent

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). Do not heat or expose to direct sunlight.

5. **Stability:** CONSED is stable to the stated expiration date when stored at the required temperature.

CALIBRATION
N/A

QUALITY CONTROL
Any product showing cloudiness, turbidity, precipitation or coloration should be discarded. The use of a positive parasite control slide for permanent staining procedures is recommended.

PROCEDURE
CONCENTRATION PROCEDURE
1. Place a PRS Concentration Funnel (or other suitable filtration apparatus) into a 15 ml polypropylene centrifuge tube. Remix the specimen thoroughly and pour the sample through the funnel into the tube. **NOTE**: A filtered specimen volume of 2 ml will be used for this concentration procedure; however, the entire fixed specimen can be filtered through the funnel with the balance of the filtered specimen poured back into the original collection tube. You may filter only 2 ml if desired. Remove and dispose of the funnel.
2. Add 8 ml of CONSED Concentration Reagent and 4 ml of ethyl acetate (or a substitute such as PRO-Clear) to the 2 ml sample in the centrifuge tube. Recap, hold thumb securely over the cap and shake. **CAUTION**: Pressure may build up in the tube during shaking. Carefully release the pressure by slowly opening the cap on the centrifuge tube away from you.
3. Place tubes in a centrifuge (with a free-swinging head) and spin for 10 minutes at 500 to 600xg. Following centrifugation four layers will develop:
   a. A top layer of mostly ethyl acetate (or substitute)
   b. An interface layer of fatty fecal debris

c. A lower solution layer
d. A pellet / sediment layer

4. With the tube upright, use the stick-end of a cotton-tipped applicator to free the fatty interface layer from the side of the tube. Holding the tube so that the pellet is always visible, carefully pour out the upper three layers, leaving the pellet undisturbed. (**NOTE**: If the pellet begins to break up, quickly upright the tube to save the entire pellet.)
5. Turn the tube to vertical position and use the cotton-tipped end of the applicator to remove debris adhering to the sides of the tube. Do not disturb the pellet.
6. Prepare the wet mount by placing 1-2 drops of the sediment pellet on to a clean glass slide and coverslip. Add one to two drops of Dobell & O'Connor's iodine solution or a dilute solution of Lugol's iodine. The proper iodine solution is necessary to yield a good result. If the iodine solution is too weak, it will not stain the organisms correctly. If the iodine solution is too strong, it will over stain the organisms and may cause clumping of the fecal material. Do not use the entire specimen pellet, saving some of the pellet for the permanent smear. Only one or two drops of this pellet are required to perform the permanent smear.

CALCULATIONS
N/A

RESULTS
In a study of more than 250 refugees, parasites were found in 152 MIF-fixed specimens (60%). The species and numbers of parasites found by direct examination were compared to those found after concentration by the CONSED procedure. Different parasites and eggs concentrated at different rates. In these studies, the results were as follows: Trophozoites of amoeba and larvae of nematodes were found to be 2 to 6 times greater in the concentrate after CONSED sedimentation than were found by direct examination; cysts of protozoa and the lighter eggs of nematodes and cestodes found were from 4 to 10 times greater depending on the species; the heavier eggs of trematodes found were from 6 to 12 times greater. When parasites or eggs were low in numbers, they were often seen on concentration when not seen on direct examination. No parasites of eggs were found in a specimen examined by direct examination that was not also found after CONSED sedimentation.

COMMENTS
1. **PRECAUTIONS**
   a. CONSED is poisonous. If swallowed, induce vomiting by giving a tablespoon of Ipecac or starch paste (flour mixed with water). Contact a physician and/or Poison Center immediately.
   b. If eyes were exposed, flush for at least 10 minutes with clean 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.

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

BIBLIOGRAPHY
1. Allen, K., Frankel, J.W., and Kwa, B., 1997. Comparison of CONSED™ and Formalin-Ethyl Acetate Methods for Concentrating Intestinal Parasites and Eggs. Am. Soc. Microbiol. Ann. Meet., Helen, GA.
2. Amin, O., 2000. "Evaluation of a new system for the fixation, concentration, and staining of intestinal parasites in fecal specimens, with critical observations on the Trichrome stain." JOURNAL of MICROBIOLOGICAL METHODS 38:127-132.
3. Bass, C.C., 1906. Uncinariasis in Mississippi. J. Amer. Med. Assn. Chic. 47:185-189.
4. Blagg, W., E.L. Schloegel, N.S Mansour, and G.I. Khalaf, 1955. A New Concentration Technic for the Demonstration of Protozoa and Helminth Eggs in Feces. Amer J. Trop. Med. Hyg. 4:23-28.
5. Faust, E.C., J.S. D'Antoni, V. Odom, M.J. Miller, C. Peres, W. Sawitz, L.F. Thomen, J. Tobie, and J.H. Walker, 1938. A Critical Study of Clinical Laboratory Technics for the Diagnosis of Protozoan Cysts and Helminth Eggs in Feces. Amer. J. Trop. Med 18:169-183.
6. Jensen, B., et al, 2000. "Comparison of Polyvinyl Alcohol Fixative with Three Less Hazardous Fixatives for Detection and Identification of Intestinal Parasites." J CLIN MICROBIOL 38(4): 1592-1598.
7. Price D.L.: Hepatic, Intestinal, and Pulmonary Trematodes. In: CRC Handbook Series on Clinical Laboratory Science. Section E: Clinical Microbiology. Vol. II, p. 168, A. von Graevenitz, Section Editor, CRC Press, Cleveland, OH, 1977.
8. Price, D.L., 1994. Procedure Manual for the Diagnosis of Intestinal Parasites, CRC Press.
9. Ritchie, L.S., 1948. An Ether Sedimentation Technique for Routine Stool Examinations. Bull U.S. Army Med. Dep. 8:326.
10. Sapero, J.J and D.K Lawless, 1953. The "MIF" Stain-Preservation Technic for the Identification of Intestinal Protozoa. Amer. J. Trop. Med. Hyg. 2:613-619.
11. Young, K.H., S.L. Bullock, D.M. Melvin, and C.L. Spruill, 1979. Ethyl Acetate as a Substitute for Diethyl Ether in the Formalin-Ether Sedimentation Technique. J. Clin. Microbiol. 10:852-853.

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

EC REP MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany CE

GLOSSARY OF SYMBOLS

LOT	Lot number / Númerode porción / Númerode 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
i	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
Σ	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

