Instructions cont.

8. Centrifuge for 2 to 3 minutes at 150 rpm. Four layers should result: a small amount of sediment in the bottom of the tube, containing the parasites; a layer of formalin; a plug of fecal debris on top of the formalin layer and a layer of ether at the top.
9. Free the plug of debris by ringing with an applicator stick and decant all the fluid. After proper decanting, a drop or two of fluid remaining on the side of the tube will drain down to the sediment. Mix the fluid with the sediment and prepare a wet mount for examination. NOTE: Tap water may be substituted for physiological saline throughout the procedure; however, saline is recommended. Some workers prefer to use 10% formalin for all the rinses (steps 4 and 5).
10. Permanent stained smears should be examined with the oil emersion objective. Although cysts and trophozoites of E. coli and trophozoites (and occasionally cysts) of E. histolytica can be detected under low or high dry magnification, structural details cannot be seen clearly except with oil immersion magnification. Also organisms may be overlooked in stained smears if low power is used to locate them, so for practical purposes, stained preparations need to be examined only with oil immersion objectives. The use of a 43X, 44X or 50X oil immersion objective will speed up the examination. However, some people find that a 5X or 6X ocular in combination with the usual oil immersion objective (97X-100X) facilitates examination. In examining the smears, light and dark (thin and thick) areas should be looked at. Usually organisms are more easily located in the thin areas, but in some preparations these portions may be poorly stained or the organisms poorly differentiated. In these cases, the thicker portions would be of more diagnostic value. Organisms are not uniformly mixed with feces, so the examiner should examine left, center and right areas. For a reliable report, a competent microscopist should examine the smear for at least 15 to 20 minutes, especially if organisms are not found.

USER QUALITY CONTROL: Use "Performance Test" procedures to inoculate the media with known parasitic organisms. Follow steps under "Procedure" to prepare the specimen.

PERFORMANCE TEST: Microorganisms being used as quality control organisms should be prepared according to the following procedures.

GROWTH PERFORMANCE: (To test growth support properties of the product)

1. Place a known parasite (protozoan cysts, helminth eggs or larvae) into 2 grams of a soft fresh stool. Mix thoroughly. Proceed as indicated in the "Instructions" section.

RESULTS:

PROCEDURE	Protozoa		Helminths	
	Trophozoites	Cysts	Eggs	Larvae
Direct Wet Mounts	+	+	+	+
Concentration	-	+	+	+
Permanent Stain	+	+	-	-
Cultivation	+	-	-	-
Uninoculated Medium	-	-	-	-

LIMITATIONS:

1. Liquid specimens should be examined within 30 minutes of passage. Semi-formed specimens should be examined within 1 hour of passage, because they may contain fragile trophozoites. If these specimens cannot be examined immediately, they should be placed in a permanent fixation.
2. Specimens containing barium, oil and other interfering substances should be rejected as unsatisfactory for examination.
3. Specimens that cannot be examined within one hour should be placed at room temperature (air conditioned) or placed in a refrigerator. Do not artificially warm the specimen.
4. Formalin and MIF solutions cannot be used as a fixative for permanent stains as these solutions do not adhere well to the slide.
5. Caution should be exercised in the handling of both PVA fixative and Formalin solutions. These solutions contain chemicals that may cause injury when they come in contact with the skin.

ANTIDOTES:

1. Neutral Formalin: If conscious, induce vomiting by giving a tablespoon full of salt in a glass of water and repeat until vomit fluid is clear. Give two (2) teaspoons full of baking soda in water. Cover eyes to exclude light. Apply artificial respiration if not breathing. Call a physician as soon as possible.

Technical Information

FORMALIN NEUTRAL
TI NO.21630

USE: This product is used in qualitative procedures for the concentration of fecal specimens for the examination of intestinal parasites.

HISTORY: In 1949, Brooke and Goldman described a method of preserving intestinal protozoa utilizing vials of polyvinyl alcohol fixative and 10% Formalin. A number of state public health laboratories are using this two vial system.

PRINCIPLE: Concentration procedures to recover protozoan cysts, helminth eggs and larvae can be performed with the formalin mixture. Neutral formalin is used for long-term preservation of helminths and protozoa.

FORMULA:

5% Formalin		10% Formalin	
Na_2HPO_4	6.10 g	Na_2HPO_4	6.10 g
NaH_2PO_4	0.15 g	NaH_2PO_4	0.15 g
Formalin (commercial formaldehyde)	400 ml	Formalin (commercial formaldehyde)	800 ml
Deionized water	7600ml	Deionized water	7200ml
pH 7.0 ± 0.2 @ 25C		pH 7.0 ± 0.2 @ 25C	

PRECAUTIONS: This product is "For In Vitro Diagnostic Use" and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers and media after their use. Directions should be read and followed carefully.

STORAGE: This product is ready for use and no further preparation is necessary. The product should be stored in its original container at room temperature. Do not freeze or overheat.

PRODUCT DETERIORATION: This product should not be used if (a) there is evidence of dehydration, (b) the product is contaminated, (c) the color has changed, (d) the expiration date has passed, or (e) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE AND TRANSPORTATION: The laboratory should provide suitable collection containers and instructions for their proper use. Specimens should be transported to the laboratory without delay and protected from excessive heat or cold.

General rules applicable to all clinical specimens:

1. Quantity of specimen should be sufficient to permit thorough examination.
2. The specimen should be collected properly and should be representative of the infected area.
3. Care should be taken to prevent contamination of specimen.
4. Specimens should be taken to the laboratory promptly.
5. Specimens should be obtained before any antibiotics are administered to the patient. If therapy was initiated prior to collection, this should be noted on the forms sent with the specimen.

For further directions on the collection and transportation of specimens, consult the chapter "Collection, Handling and Processing of Specimens" in the ASM Manual, 3rd edition, 1980.

PROCEDURE: The usual clinical microbiological equipment is required for procedures involving this product. The media and equipment required will depend on the identification scheme employed by the microbiologist.

INSTRUCTIONS:

1. Due to the intermittent passing of certain parasites from the host and the limitation of the diagnostic techniques available, the examination of multiple specimens is recommended. For example, the nematode species shed eggs almost daily whereas the protozoa fluctuate from day to day intervals. The stool should be passed into a dry container and the introduction of urine into the container is to be avoided.
2. A ratio of one part stool to 3 parts formalin should be attained for complete fixation and preservation. Mix the specimen thoroughly with the solution. Let stand for a minimum of 30 minutes for adequate fixation.
3. Place two layers of gauze in funnel (funnel not absolutely necessary) and strain vial contents through the gauze into a 15ml centrifuge tube.
4. Add saline solution almost to the top of the tube and centrifuge for 2 minutes at approximately 1500 rpm.
5. Decant and resuspend the sediment; add saline solution almost to the top of the tube and centrifuge again for 2 minutes at 150 rpm. This second wash may be eliminated if the supernatant fluid after the first wash is light tan or clear.
6. Decant and resuspend the sediment on the bottom in 5-10% formalin. Fill tube half full only. If the amount of sediment left in the bottom of the tube is very small in step 6, do not add ether in step 7, merely add the formalin, spin, decant and examine the remaining sediment.
7. Add approximately 3ml of ether (do not use around open flames) or ethyl acetate, stopper and shake for 30 seconds. Hold the tube so that the stopper is directed away from your face, since the pressure from shaking the formalin-ether mixture may cause the stopper to pop out of the tube.

remel
REGIONAL MEDIA LABORATORIES INC.
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CATALOG NUMBER(S):

21-630	Formalin (5%) Neutral (Vial w/Spatula) - 15ml	12/ca
21-631	Formalin (5%) Neutral/MIF	6 sets/ca
21-632	Formalin (5%) Neutral/PVA-Fixative	6 sets/ca
21-633	Formalin (5%) Neutral/PVA-Modified Fixative	6 sets/ca
21-634	Formalin (5%) Neutral/Empty Vial	6 sets/ca
21-695	Formalin (5%) Neutral/PVA Fixative/Cary Blair w/Ind.	4 sets/ca
21-696	Formalin (5%) Neutral/PVA Fixative/Glycerol Saline (Buff.)	4 sets/ca
21-715	Formalin (5%) Neutral/PVA-Mod. Fixative/Cary Blair w/Ind.	4 sets/ca
21-716	Formalin (5%) Neutral/PVA-Mod. Fixative/Glycerol Saline (Buff)	4 sets/ca
21-640	Formalin (10%) Neutral (vial w/Spatula) - 15ml	12/ca

Revised
09/26/85
cji