Instructions cont.

8. Centrifuge for 2 to 5 minutes at 150 rpm. Four layers should result: a small amount of surface debris at the top, containing the parasites; a layer of formalin; a plug of feral debris at the bottom of the tube, containing the parasites; a layer of formalin; a plug of feral debris on top of the formalin layer and a layer of ether at the top.

9. Incubate the plug of debris by running an aspirator stick and swab all the fluid.

10. After proper decanting, a drop of two or all fluid remaining one of the three tubes is drawn and the liquid is allowed to precipitate the sediment. The fluid with the sediment is ready to be examined. The sediment is ready for examination.

11. Syringe the fluid to 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).

12. Permanent stained smears will be examined with the oil immersion objective. Although ciphers and trichomeos of E. coli and trichomeos (and occasionally cysts) of E. histolytica can be seen under low or high magnification, structural details cannot be seen clearly 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 should be examined only with oil immersion objectives. The use of a 40X, 40X or 50X oil immersion objective will speed up the examination. However, some people find that a 40X oil immersion combination with the usual oil immersion objective (90X-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 organism poorly differentiated. In these cases, the thicker portions will be of more diagnostic interest. Organisms are not uniformly stained with eosin. The examiner should examine thick, light, and thin areas.

For a reliable report, a competent microscopist should examine the smear for at least 5 to 10 minutes, especially if organisms are not found.

USER QUALITY CONTROL: See "Performance Test" procedures to inoculate the media with known pathogens. Perform quality control at the test site. Follow steps under "Procedure" to prepare the specimen.

INTERPRETATION OF RESULTS: Microorganisms being used as quality control organisms should be prepared following the procedure. Growth performance is indicated in the "Interpretation" section.

LIMITATIONS:

1. Nematode cysts should be examined within 30 minutes of passage. The presence of cysts may be observed within 1 hour of passage, because they are more easily seen than trophozoites. These cysts may not be observed in any tissue, so they should be present in a permanent fixation.

2. Parasites containing trophozoites, cysts, and other trophozoites may be rejected as insufficient, and their number may be reduced. If these cysts are not observed immediately, they should be present in a permanent fixation.

3. Specimens that cannot be examined within 1 hour should be diluted and placed in a refrigerator. Do not allow any specimen to be stored above 4°C.

4. Formalin and NBF solutions cannot be used as a fixative for permanent stains as these solutions do not adhere well to the slides.

5. Caution should be exercised in the handling of both NBF and Formalin solutions. These solutions contain chemicals that may cause injury when they come in contact with the skin.

ANTISEPTIC:

1. Formalin: If necessary, induce vomiting by giving a tablespoon full of salt in a glass of water and repeat until vomit fluid is clear. Give two (2) teaspoonful 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

10% Formalin

FORMALIN

Formalin (formaldehyde) is used as a fixative and 10% Formalin. A number of state public health laboratories are using this as a test system.

PRINCIPLE: Concentration procedures to recover protozoan cysts, helminth eggs and larvae can be performed by stirring a sterile solution mixture of Formalin with neutral Formalin. Neutral Formalin is used for long-term preservation of helminth and protozoan organisms.

FORMULA

1. 10% Formalin

2. NBF (Neutral Formalin)

3. Formalin (formaldehyde) is used as a fixative and 10% Formalin. A number of state public health laboratories are using this as a test system.

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

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

SPECKLE COLLECTION, STORAGE AND TRANSPORTATION: The laboratory should provide suitable collection containers and instructions for their proper use. Specimens should be transported to the laboratory by the fastest means possible. Specimens may be kept frozen in a refrigerator as long as they are not exposed to extreme cold or heat.

General rules applicable to all clinical specimens:

1. Specimens should be collected properly and should be representative of the infected tissue.

2. Care should be taken to prevent contamination of the specimen.

3. The specimen should be taken to the laboratory promptly.

4. Specimens should be brought to the laboratory promptly. If this is not possible, the specimen should be stored at 4°C to 8°C.

For further details on the collection and transportation of specimens, consult the chapter "Collection, Handling, and Processing of Specimens" in the 43rd Annual, 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.

INSTRUCTION:

1. Use the intermittent passage of certain parasites from the host and the limitation of the diagnostic techniques available, the examination of multiple specimens is recommended. For example, the asexual parasite is used with fresh material, whereas the protozoa, which live in the host, may be used with a single specimen.

2. A ratio of one part stool to 3 parts formalin should be obtained for complete digestion and gentle stirring of the specimen thoroughly with the solution. Let stand for a minimum of 5 minutes for adequate fixation.

3. Place two layers of glassine in formalin (formaldehyde) thoroughly and stain with vital dyes containing the glassine in a 5% formalin solution. Let stand for a minimum of 3 minutes.

4. Add sterile water to the top of the tube and centrifuge for 2 minutes at approximately 1500 rpm.

5. Decant and resuspend the sediments; add saline solution to the top of the tube and centrifuge for 2 minutes at approximately 1500 rpm.

6. Decant and resuspend the sediments on the semi-infective medium. Fill tubes half full only. If the amount of sediments left in the bottom of the tube is very small in step 5, add saline solution to the sediment and centrifuge for 2 minutes at approximately 1500 rpm.

7. Decant and resuspend the sediments on the semi-infective medium. Fill tubes half full only. If the amount of sediments left in the bottom of the tube is very small in step 5, add saline solution to the sediment and centrifuge for 2 minutes at approximately 1500 rpm.

8. Decant and resuspend the sediments on the semi-infective medium. Fill tubes half full only. If the amount of sediments left in the bottom of the tube is very small in step 5, add saline solution to the sediment and centrifuge for 2 minutes at approximately 1500 rpm.

REMEMBER: The specimen should be taken to the laboratory promptly. If this is not possible, the specimen should be stored at 4°C to 8°C.

9. Decant and resuspend the sediments on the semi-infective medium. Fill tubes half full only. If the amount of sediments left in the bottom of the tube is very small in step 5, add saline solution to the sediment and centrifuge for 2 minutes at approximately 1500 rpm.
BIBLIOGRAPHY:

CATALOG NUMBER(S):

21-630  Formalin (5%) Neutral (Vial w/Spatula) - 15a  12/case
21-631  Formalin (5%) Neutral/PFA Fixative  6/case
21-632  Formalin (5%) Neutral/PFA-MePfixative  6/case
21-633  Formalin (5%) Neutral/PFA-MePfixative/Cryoblair w/Ind.  6/case
21-634  Formalin (5%) Neutral/PFA Fixative/Cryoblair w/Ind.  4/case
21-635  Formalin (5%) Neutral/PFA Fixative/Glycerin Osmoline (Buff.)  4/case
21-636  Formalin (5%) Neutral/PFA-MePfixative/Glycerin Osmoline (Buff.)  4/case
21-637  Formalin (5%) Neutral/PFA-MePfixative/Cryoblair w/Ind.  4/case
21-638  Formalin (5%) Neutral/PFA-MePfixative/Cryoblair w/Ind. (Buff.)  4/case
21-639  Formalin (10%) Neutral (Vial w/Spatula) - 15a  12/case

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