FIXATIVES FOR SPECIMENS CONTAINING INTESTINAL PARASITES

PRODUCT:

Vial:[a]

Formalin, 10%, item no. E2000, E2020
Polyvinyl Alcohol,item no. E9000, E9020
Polyvinyl Alcohol, Modified, item no. E8000, E8020
Polyvinyl Alcohol, Zinc Sulfate, item no. E9906, E9907
Sodium Acetate, Acetic Acid, Formalin,item no. E3000, E3010
Empty Clean Vial, item no. E1050, E1060

[a]see catalog for ordering options

PURPOSE:

Each of these fixatives, when properly mixed with a clinical specimen, will preserve diagnostic stages of intestinal parasites for later examination. The empty vial is used to transport unpreserved stool specimens for parasite examination (within 30 minutes of collection), occult blood, and fat determination.

PRINCIPLES:

Specimens for parasitic examination should be properly collected and then examined within 30 minutes of collection before some of the diagnostic stages are lost. It is not always possible to examine specimens within the first hour, and fixative reagents were developed to overcome this limitation. These fixatives, when properly used, preserve diagnostic stages and characteristics for some time without adversely affecting them.[5,6]

Each fixative has its limitations.[5,6] Formalin, which preserves cysts, eggs, and larvae, can be used in examinations of wet mounts or concentration procedures but not for permanent smear preparations. Polyvinyl Alcohol (PVA) and Polyvinyl Alcohol, Modified (m-PVA), and Zinc Sulfate Polyvinyl Alcohol (Zinc-PVA), which preserve trophozoites, cysts, and eggs, are used for making permanent smears for staining procedures but are not routinely used for examinations of wet mounts and are not applicable to concentration procedures.[1,2,3,4]

For many years Schaudinn and Polyvinyl Alcohol (PVA) fixatives with mercuric chloride ($HgCl_2$) base have been used to preserve stool specimens for the recovery and identification of intestinal parasites.[1] During the past 10 years the question of mercury disposal has been raised by clinical laboratories. In the m-PVA formulation, the $HgCl_2$ has been replaced with copper sulfate, eliminating the mercury hazard. A study[3] of $ZnSo_4$ found this mercury substitute to be an acceptable alternative, and can also be used with trichrome stain. Sodium Acetate, Acetic Acid, Formalin (SAF), which preserves trophozoites, cysts, eggs, and larvae, can be used for direct examinations, concentration procedures, and smears for permanent staining procedures.[5] With the substitute fixatives and methods available at this time, Garcia, et al, is recommending the use of Zinc-PVA stained with trichrome as a replacement for the current use of mercury-containing PVA stained with trichrome with a second, equally acceptable, choice being the SAF fixative coupled with iron hematoxylin stain.

The Empty Clean Vial can be used to send unpreserved stool specimens to the lab for several determinations. If the specimen will be examined within 30 minutes of collection, parasite examination and bacterial cultures can be performed. The specimen can also be used for occult blood and fat determination.

FORMULAS:

Approximate ingredients.

(1) Formalin, 10%:

Disodium Phosphate	760.0 mg
Monosodium Phosphate	15.0
Formaldehyde, 40%	100.0 ml
Water, Deionized	900.0

(2) PVA:

Polyvinyl Alcohol	50.0 g
Mercuric Chloride, Sat. Sol.	45.0
Ethanol, Absolute	310.0 ml
Glycerol	15.0
Glacial Acetic Acid	50.0
Water, Deionized	625.0

(3) m-PVA:

Polyvinyl Alcohol	53.0 g
Copper Sulfate	16.0
Ethanol, Absolute	317.0 ml
Glycerol	16.0
Glacial Acetic Acid	53.0
Water, Deionized	633.0

(4) Zinc-PVA:

Polyvinyl Alcohol	53.0 g
Zinc Sulfate	89.0
Ethanol, Absolute	317.0 ml
Glycerol	16.0
Glacial Acetic Acid	53.0
Water, Deionized	614.0

(5) SAF:

Sodium Acetate	15.0 g
Glacial Acetate Acid	20.0 ml
Formaldehyde, 40%	40.0
Water, Deionized	925.0

PRECAUTIONS: *

For in vitro diagnostic use only. Observe all safety precautions consistent with the hazard(s) stated on the product label and/or Material Safety Data Sheet. Fixatives should not be used if there are signs of deterioration or if the expiration date has passed. Avoid contact with eyes, skin, and mucous membranes. If contact occurs, flush area with running water. If irritation continues, contact a physician. These formulations are poisonous. **DO NOT TAKE INTERNALLY**. If swallowed, contact a physician or poison control center immediately. Do not breathe the vapors directly from an opened PVA vial.

Storage: Upon receipt store at 10-30°C away from direct light.

Limitations: A small amount of sediment may form on the vial bottom. It will not interfere with the examination. Avoid cold storage temperatures. PVA will show some sediment, varying from lot to lot, but will become excessively sedimented if exposed to cold. Warm excessively sedimented PVA prior to use. Sediment does not affect the fixation properties.

Inadequate fixation or use of fixatives beyond their expiration date will cause significant changes in the resulting stained smear. False-negative examinations may occur if too much or too little specimen is used in the concentration procedure.

Entamoeba coli cysts may not fix well in the SAF procedure, making it difficult to see them on the stained smear. Doubling the fixing time will improve on their appearance.

Although PVA, m-PVA, and Zinc-PVA fixative can be used for sputum, abscess aspirates, sigmoidoscopic materials, and urogenital materials, it is best to prepare direct smears from those specimens and then place them into Schaudinn or some other fixative for transporting to the laboratory. This fixative is primarily used for protozoan cysts and helminth eggs.

Iospora cysts do not survive fixation in PVA.

PROCEDURE: *

Specimen Collection: Collect the stool specimen in a clean, dry, and waterproof container. Do not contaminate it with urine. Immediately fill the vial(s) of choice to the fill line on the label (1 part of the specimen with 3 parts of the fixative) and mix thoroughly.

If the specimen can be taken to the laboratory immediately upon collection and processed, a preservative is not needed. Information on specimen collection can be found in standard reference material on the subject.[1,4] The unpreserved specimen should be examined within 30 minutes of collection.

Methods of Use: Depending on the fixative used, some or all of the following procedures can be used for examining clinical specimens. For complete descriptions and related information of the following procedures, refer to reference materials in clinical parasitology.[5,6]

Wet Mount Examinations (Formalin, SAF):

1. Saline mount. Place a drop of physiological saline on a glass microscope slide and emulsify a small portion of the preserved specimen in it. Overlay the suspension with a cover slip. One should be able to read newspaper print through the slide and suspension. Microscopically examine smear for the presence of parasites.
2. Iodine mount. Same procedure as for saline, except a drop of a one percent solution of iodine is used instead of the saline. The iodine will highlight internal structures of parasites that may be present in the specimen making them more recognizable.

Concentration Procedure (Formalin, SAF):

1. Stir the preserved specimen to resuspend it.
2. Strain the preserved specimen through 1 or 2 layers of narrow mesh gauze into a centrifuge tube (10 ml).
3. Centrifuge 1-2 minutes at 2000-2500 rpm.
4. Decant supernatant. Repeat steps 1-3 if necessary until 0.5-1.0 ml of sediment is remaining, resuspending sediment with saline.
5. Mix 10 ml of fresh water or saline with the sample and shake vigorously.
6. Centrifuge the water or saline suspension for 1 minute at 1,500 rpm. Decant the supernatant.
7. Repeat steps 7 and 8 until the supernatant is clear.
8. Add 9 ml of 10% neutral formalin to the sediment.
9. Add 4 ml of diethyl ether to the sediment/formalin suspension. Stopper the tube and shake vigorously for 30 seconds in an inverted position.
10. Centrifuge the suspension for 1 minute at 1800 rpm. Four layers should result:
    a. Ether layer at the top.
    b. Plug of debris.
    c. Formalin solution.
    d. Sediment.
11. Ring the plug (second layer) with an applicator stick and decant all but the sediment and a small amount of liquid. Remove debris from the side of the tube using a cotton-tipped applicator.
12. Mix the sediment with the remaining liquid and add several drops of neutral formalin to neutralize the ether.
13. Make a wet mount as described above, using the sediment, and examine microscopically for parasites. Sediment may be stored several days by adding 1-2 ml of formalin.

NOTE: Comparable results have been reported substituting ethyl acetate for diethyl ether. Ethyl acetate is less flammable and, therefore, less dangerous.

Permanent Smear Procedures (PVA, m-PVA, Zinc-PVA, SAF):

PVA, m-PVA, Zinc-PVA

1. To collect some of the specimen, dip an applicator stick into the preserved specimen.
2. Place a drop or two of specimen on a glass slide and then spread (do not smear) a thin film over one third of the glass surface. Make sure the smear is not too thick and that it extends to the sides of the slide to reduce peeling.
3. Allow the smear to air dry thoroughly, preferably overnight at 37°C, before staining.
4. Dried films can be held for several weeks before being stained.
5. Prior to staining, the dried PVA films are placed in 70% alcohol and iodine to remove the mercuric bichloride. m-PVA and Zinc-PVA smears to not require this step.
6. Stain the smear with the appropriate parasitological stain procedure. Staining techniques can be found in standard texts on clinical parasitology. See Parasitology Stains, Data #915.
7. Microscopically examine smears for the presence of parasites.

SAF

1. Place a drop of Mayer's Albumin on a clean glass slide.
2. Mix a small portion of the sediment from the centrifugation button or directly from the SAF-preserved specimen with the drop of albumin.
3. Spread the mixture over the slide to form a thin film. If the film is too thick it may have a reddish hue to it.
4. Allow the smear to dry at room temperature until it becomes tacky, usually from 5-10 minutes.
5. Immerse the slide in 70% ethyl alcohol until the albumin coagulates, usually 25-35 minutes.
6. Stain the smear using the procedure of choice. Treatment of these smears with an iodine/alcohol solution prior to staining is not necessary because there is no mercuric chloride present.
7. Microscopically examine the stained smear for the presence of parasites.

Interpretation: Refer to standard reference material and to Parasitology Stains, Data #915.

Other Materials Required but Not Supplied: Standard microbiological supplies and equipment such as centrifuge, microscope, slides, and stains are not provided.

QUALITY CONTROL: *

Stock cultures of parasites are used to test the effectiveness of the fixative. Permanent smears or wet mounts are examined for the preservation of the parasites used and for appropriate staining characteristics.

The morphological characteristics of parasites should appear as defined for the staining procedures used. See standard references.

User Quality Control: Check for signs of deterioration.

BIBLIOGRAPHY:

1. Brooke, M.M., "PVA Fixative Technique in Laboratory Confirmation of Amebiasis," *Triangle*, 4:326-335,1960.
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3. Garcia, L. S., et al., "Evaluation of Intestinal Protozoan Morphology in Polyvinyl Alcohol Preservative: Comparison of Zinc Sulfate—and Mercuric Chloride—Based Compounds for Use in Schaudinn's Fixative," *J. Clin. Microbiol.*, 31:307-310, 1993.
4. Horen, W. P., *J. Clin. Microbiol.*, 1:204-205, 1981.
5. Koneman, E. W., et al., *Color Atlas and Textbook of Diagnostic Microbiology*, 2nd ed., J. B. Lippincott, St. Louis, 1983, pp. 567-629.
6. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 3rd ed., American Society for Microbiology, Washington, D. C., 1980.
5. Yang, J., and T. Scholten, "A Fixative for Intestinal Parasites Permitting the Use of Concentration and Permanent Staining Procedures," *Am. J. Clin. Pathol.*, 67:300-304, 1977.

*For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

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