Technical Information

PVA MODIFIED FIXATIVE
TI No. 21710

CONTENTS OF O&P KIT

USE: This medium is used in qualitative procedures for the preservation and permanent staining 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.[1] PVA Modified Fixative was developed by P. Horen to replace the potential toxicity of the original fixative containing mercuric chloride.[2]

PRINCIPLE: Polyvinyl alcohol is a synthetic water-soluble polymer of vinyl alcohol which is stable over a long period of time. PVA is effective as an embedding medium for tissues, a mounting medium for insects and fungi, and as a means of reducing motility of paramecia and other organisms. Copper sulfate is used in place of mercuric chloride in this formulation because it eliminates the hazard of accidental poisoning due to ingestion of the medium without interfering with the desired properties of the product.

CLASSICAL FORMULA:*

Polyvinyl alcohol powder	50.0 g	Reagent alcohol	310.0 ml
Glycerol	15.0 ml	Glacial acetic acid	50.0 ml
Cupric sulfate	12.5 g	Deionized water	625.0 ml

*Adjusted as required to meet performance standards.

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 until used. Do not freeze or overheat. Do not incubate prior to use.

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.

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:

A. Collection of stool specimens:
   1. Due to the intermittent passing of certain parasites from the host and the limitations of the diagnostic techniques available, the examination of multiple specimens is recommended. 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, (3-5 spatulas) to 3 parts PVA should be attained for complete fixation and preservation. Mix the specimen thoroughly with the solution.
   3. PVA-fixed specimens: Pour some of the PVA mixture onto a paper towel and allow to stand for 3 minutes to absorb out PVA. With an applicator stick, apply some of the preserved specimen over the surface of a microscope slide, taking care to extend the smear to the edges of the slide. The films are allowed to dry thoroughly for several hours or overnight either in a 35-37C incubator or at room temperature. The films should dry thoroughly to prevent the material from washing off the slide during staining. Slides may be left unstained up to 2 months.
   4. Stain slides using Wheatley Trichrome Stain or the Hematoxylin Stain procedure.

B. Collection of specimens other than feces:
   1. Sputum: Pulmonary amebiasis and echinococcosis may be detected in the sputum and for this reason should be examined for trophozoites immediately and preserved in PVA fixative for subsequent staining. When examination is delayed, the sputum may be preserved in 5% neutral formalin.
   2. Vagina: Specimens to be examined immediately should be placed in saline solution. Prepared smears should be placed in Schaudinn's fixative. If the examination is to be delayed, the material should be placed in PVA fixative.
   3. Anal specimens for *Enterobius vermicularis* should be collected by a tape-slide preparation or Vaseline-paraffin swab between the hours of 9PM and midnight or in the early morning before defecation or bathing.
   4. Urine: Urine specimens are utilized in the diagnosis of *Trichomonas vaginalis* and *Schistosoma haematobium* infections. Examine the most fresh urine specimens possible for *Trichomonas vaginalis* because the flagella becomes immobile in older specimens. The optimum urine specimen for *Schistosoma* is that passed shortly after the noon hour. Multiple specimens are recommended.
   5. 0.85% Saline solution should be used as a diluent in the examination of duodenal films and sediment by centrifugation. Since *Giardia* trophozoites are lysed in bile, the specimen should be preserved in formalin unless examined immediatley.

MATERIALS REQUIRED BUT NOT SUPPLIED: (1) Loop sterilization device, (2) Inoculating loop, swab, collection containers, (3) Incubators, anaerobic jars or candle jars, (4) Supplemental media, (5) Quality control organisms.

USER QUALITY CONTROL:

1. Inoculate a known parasite into approximately 1/4 teaspoon of soft, fresh stool. Add this mixture to 10ml of PVA.
2. After 30 minutes of fixation, pour the stool-PVA mixture onto paper towels to absorb out the excess PVA.
3. The material can then be smeared onto several slides, allowed to dry thoroughly (30 to 60 minutes at room temperature), and stained.
4. Examine microscopically. If the parasite appears well fixed and typical morphology is visible, one can assume that any intestinal protozoa placed in the same lot number of PVA fixative would also be well fixed provided the fecal sample was fresh and fixed within recommended time limits.

LIMITATIONS:

1. Specimens containing barium, oil and other interfering substances should be rejected as unsatisfactory for examination.
2. Specimens that cannot be examined within 1 hour should be placed at room temperature (air conditioned) or placed in a refrigerator. Do not artificially warm the specimen.
3. Entamoeba coli cysts are difficult to fix properly and may be difficult to identify on the stained slide. For this reason, it is possible to have fixatives that meet quality control criteria and yet do not always yield good morphology for this particular organism. Use of a longer fixation time (60 minutes) sometimes produces better morphology after staining.

BIBLIOGRAPHY:

1. Brooke, M.M. and M. Goldman, Polyvinyl Alcohol-Fixative As A Preservative and Adhesive For Protozoa in Dysenteric Stools and Other Liquid Materials, Journal Lab. Clinc. Med., 34 11:1554, 1949.
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