Preservation of specimens:

Is necessary when stool specimens cannot be examined within the prescribed time interval. Various preservatives are available (see table), with the two most commonly

General preservatives for stool specimens

Preservatives	Advantages	Disadvantages
10 % Formalin	1-All purpose fixative 2-Easy to prepare 3-Good preservation of morphology of helminth eggs, larvae, protozoan cysts and coccidia 4-Suitable for concentration procedure and UV fluorescence microscopy 5-Suitable for acid-fast, safranin and chromotrope stains 6-Compatible with immunoassay kits and UV fluorescence microscopy	1-Not suitable for some permanent smears stained with trichrome 2- Inadequate preservation of morphology of protozoan Trophozoites 3-Can interfere with PCR especially after extended fixation time

MIF (Merthiolate-iodine-formaldehyde)	1-Components both fix and stain organism 2-Easy to prepare 3-Long shelf life 4-Useful for field surveys 5-Suitable for concentration procedure	1-Not suitable for some permanent smears stained with trichrome 2-Inadequate preservation of morphology of protozoan Trophozoites 3-Iodine interferes with other stains and fluorescence 4-Iodine may cause distortion of protozoa.
LV-PVA (Low viscosity polyvinyl-alcohol)	1-Good preservation of morphology of protozoan Trophozoites and cysts 2-Easy preparation of permanent smears stained with such as trichrome (solution both preserves organisms and makes them adhere to slides) 3-Preserved samples remain stable for several months	1-Inadequate preservation of morphology helminth eggs ,larvae coccidia and microsporidia 2-Contains mercuric chloride 3-Difficult and expensive to dispose of 4-Difficult to prepare in the laboratory 5- Not suitable for concentration procedures
		6-Can not be used with immunoassay kits 7-Not suitable for acid-fast, safranin and chromotrope stains

SAF (sodium acetate-acetic acid-formalin)	1-Suitable for both concentration procedure and preparation of permanent stained smears 2-Easy to prepare 3-long shelf life 4-Suitable for acid-fast safranin and chromotrope stains 5-Compatible with immunoassay kits	1-Requires additive (e.g. albumin-glycerin) for adhesion of specimens to slides 2-Permanent stain not as good as with PVA or Schaudinn´s fixative
Schaudinn´s fixative	1-Good preservation of morphology of protozoan Trophozoites and cysts 2-Easy preparation of permanent stained smears	1-Less suitable for concentration procedures 2-Contains mercuric chloride 3-Inadequate preservation of morphology of helminth eggs ,larvae, coccidia and microsporidia 4-Poor adhesion of liquid or mucoid specimens to slides
Modified PVA copper or zinc	1-Permanent smears can be made and stained with trichrome 2-Zinc is preferred over copper 3-No mercuric chloride	1-Staining not consistent 2-Organism morphology may be poor 3-Copper-morphology of cysts and Trophozoites is poor 4-Zinc-better morphology but not comparable to LV-PVA

Because 10% formalin and PVA have complementary advantages, it is recommended that the specimen be divided and preserved in both types of preservatives two-vials kits are available for this purpose. Preserved specimens can be stored for several months. MIF Stain –preservative solution :

Stock MIF solution :

Prepared from -:

Distilled water ----------250 ml

Tincture of merthiolate -------200 ml

Formaldehyde ----------25 ml

Glycerin ----------5 ml

This solution is stored in brown glass bottles. For use it is combined with fresh Lugol's solution (not over 1 week old) in the following manner :

-١Measure 2.35vml stock MIF solution into a small test tube and stopper with a cork .

-٢Measure 0.15 ml Lugol's solution into a second tube and close with a rubber stopper .

The two solutions are combined immediately before adding the fecal specimen. The amount of fecal material to be added to this volume of preservative should be about 0.25 g. The specimen is broken up in the MIF solution and mixed thoroughly . The specimen may be examined immediately or stored in a well-stoppered tube , it will retain a good stain for some months . After storage , it will be found that most protozoa and helminth eggs floats in the upper layers of sedimented feces . A drop of mixed supernatant fluid and feces is withdrawn , placed on a slide and covered with a coverslip .

PVA Fixative solution :

The original PVA fixative consist of a mixture of polyvinyl alcohol , glycerin, glacial acetic acid and Schaudinn´s solution . it has the disadvantages of containing mercuric chloride , with consequent disposal problems . Accordingly it has been modified by the substitution of copper or zinc for the mercury . Although posing less problems of disposals , these modified solutions give precise preservation of intestinal protozoan morphology than does the mercury- containing solution .

PVA solutions of these sorts are obtainable in a wide variety of commercial kits as well as in bulk . If obtained in bulk , it is convenient to dispense the PVA solution in screw-capped vials , in approximate 5 ml quantities . To this volume fixative about 1 g formed feces(somewhat more of liquid) may be added. Formed feces must be broken up and mixed thoroughly with the preservative with the preservative solution.

The solution preserves both Trophozoites and cysts of protozoa , most eggs are recognizable after PVA preservation but it is often advisable to include in the packet a second vial containing 10% formalin to allow for concentration for eggs from another aliquot of feces. Protozoa retain their staining quality for at least a month. If a series of stool specimens is desired , the patients may preserve them immediately after passage and bring them all in at one time for processing .

To appear slides for staining shake the preserved specimen well or mix using applicator sticks. Pour some of the PVA mixture onto blotting paper allow excess PVA to be absorbed. Apply the preserved stool to the slide and dry for 2 hours at 37℃ or overnight at room temperature. The slides may then be stained with trichrome , but the dry slides may be kept unstained for long periods of time without loss of morphology .

SAF Fixative solution

Like PVA , the SAF fixative-preservative may be used for the preservation of material ,which can be then concentrated by the formal-ethyl acetate technique or made into permanent stained smears. The fixative is more fluid than PVA and the preserved specimen must be centrifuged after staining through gauze and the sediment used to prepare smears for staining , adherence to the glass slide may be improved if the slide is coated with albumin . After drying the slides may be placed in 70% alcohol . The fixative solution is made up as follows :

Sodium acetate ----------1.5 gm

Acetic acid glacial --------2 ml

Formaldehyde 40%-------4 ml

Distilled water ----------92.5 ml

Schaudinn´s solution

A constituent of the original PVA solution is also used as a fixative and preservative for fresh stool specimens or scrapings from the intestinal mucosa and as a constituent of the original PVA it carries the same disposal problems.

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