((Preparation of Solutions))

1. Polyvinyl Alcohol(PVA):

Composition

Schaudinn's stock solution 93.5 ml

Glycerol 1.5 ml

Glacial acetic acid 5.0 ml

Polyvinyl alcohol 5 g

Advantages:

- Fixative for all purpose.
- Has a long shelf life (months to years) in tightly sealed containers at room temperature.
- The preservation of the two stages of protozoa (trophozoite and cyst) is excellent, and also suitable for helminthes eggs and larvae.
- Compatible with immunoassay kits and UV fluorescence microscopy
- The PVA is a plastic resin that serves as adhesive for the stool material, When the stool-PVA mixture is spread onto the glass slide, it adheres because of the PVA component.
- The greatest advantage of this fixative is that a permanent stain can be prepared from stool specimen preserved by PVA, giving excellent result with trichrome staining.
- Commercially available from a number of sources.

Disadvantages:

- Contains mercury compounds (Schaudinn's fixative), which is highly toxic to both man and the environment, and must be disposed of as toxic waste.
- Concentration methods can't performed from the specimen preserved by PVA.
- Difficult to prepare in the laboratory.
- Some organisms (Trichuris trichiura eggs, Giardia lamblia cysts, Isospora belli oocysts) are not concentrated as well from PVA , and morphology of some ova and larvae may be distorted.

- Can interfere with PCR, especially after extended fixation time

	Formalin	PVA
Toxicity	+/-	+++(duo to Hg)
Shelf life	Long (months)	Long (months/years)
Preparation	Easy	Difficult
Quality of fixation	Egg: ++	Egg: ++
	Cyst: ++	Cyst: +++
	Troph's: +/-	Troph's: +++
Formalin ether concentration	Possible	Not possible
Permanent stained smear	Not possible	Only Trichrome

2. Schaudinn's fixative

Saturated solution of mercuric chloride (HgCl2)

Procedure:-

1. Dissolve 10 g HgCl2 in 100 ml warm (not boiling) distilled water, Leave to cool (mercuric chloride crystals deposit)
2. Filter off the clear supernatant. 3. Store in a sealed glass bottle until use. 4. Label the bottle (it is very important).

Schaudinn's stock solution

Composition:

- Saturated solution of mercuric chloride (HgCl2) 2 volumes
- 95% ethanol 1 volume
- Mix well.

Schaudinn's working solution

- Immediatly before use, add (5ml) glacial acetic acid to (95 ml) of solution in a Coplin jar & mix well.
  (This mixture is stable for tow weeks).

Principle :

Schaudinn's fixative adheres the fecal material to the slide and maintains the staining integrity of protozoan trophozoites and cysts found in specimens.

Advantages:

- Desgined to be used for the fixation of slides prepared from fresh fecal specimens or samples from the intestinal mucosal surfaces.
- Prepared slides can be stored in the fixative for up to a week without distortion of protozoan organisms.
- Easily prepared in the laboratory.
- Available from a number of commercial suppliers.

Disadvantages:

- Not recommended for use in concentration techniques.
- Has poor adhesive properties with liquid or mucoid specimens.
- Contains mercury compounds (mercuric chloride), which may cause disposal problems.

3.The Buffered Methylene Blue (BMB) Wet Mount:

The Buffered Methylene Blue stain has been used successfully for staining protozoan trophozoite in wet preparations.

a) Stock reagents.

Solution A:- acetic acid

- Glacial acetic acid. (1.2 ml)
- Distilled water (98.8ml)

In a clean stoppered glass bottle add the glacial acetic acid to about 50 ml of distilled water mix well then complete the volume of distilled water and store.

Solution B:- sodium acetate

- Sodium acetate 1.64 gm
- Distilled water 100 ml

In a clean stoppered glass bottle put the sodium acetate (1.64gm), dissolve in distilled water. Mix well and store.

(b) Working solution :

Solution A ⟶ 46.3 ml

Solution B ⟶ 3.7 ml

Methylene Blue powder. ⟶ 3.0 gm

Mix the ingredients well. This solution should be prepared just prior to use for optimum results.

Procedure :-

1. Should be prepared each time amoebic trophozoites are seen in a saline wet mount, or when their presence is suspected.
2. Using an applicator stick, mix a small amount of fecal material with a drop of Buffered Methylene on a slide.
3. Wait for (5 minutes) to allow the stain to penetrate the trophozoites. It will overstain the trophozoites in 30 minutes.

•Advantages:

- BMB stain is a pproparaite only for fresh unpreserved specimens.
- BMB stains **amoebic trophozoites.**
- BMB stain live organism only.

Disadvantages:

- Not Stain ameobic cysts, flagellates trophozoites.
- It isn't used on preserved samples in which the organism have been killed.