

**Preservatives used in Diagnostic Parasitology (Intestinal Tract Specimens)**

<b>Preservative</b>	<b>Concentration</b>	<b>Permanent Stained Smear</b>	<b>Immunoassays (<i>Giardia lamblia</i>, <i>Cryptosporidium</i> spp.)</b>	<b>Comments</b>
5% or 10% Formalin	Yes	No	Yes	EIA, FA, cartridge
5% or 10% Buffered Formalin	Yes	No	Yes	EIA, FA, cartridge
MIF	Yes	Polychrome IV stain	ND	No published data
SAF	Yes	Iron Hematoxylin	Yes	EIA, FA, cartridge
PVA <sup>1</sup>	Yes	Trichrome or Iron Hematoxylin	No	PVA interferes with immunoassays
PVA-Modified <sup>2</sup>	Yes	Trichrome or Iron Hematoxylin	No	PVA interferes with immunoassays
PVA-Modified <sup>3</sup>	Yes	Trichrome or Iron Hematoxylin	Some, but not all.	PVA interferes with immunoassays
Single Vial Systems <sup>4</sup>	Yes	Trichrome or Iron Hematoxylin	Some, but not all.	Check with the manufacturer
Schaudinn's (without PVA) <sup>1</sup>	No	Trichrome or Iron Hematoxylin	No	Mercury interferes with immunoassays

<sup>1</sup> These two fixatives use the mercuric chloride base in the Schaudinn's fluid; this formulation is still considered to be the "gold standard", against which all other fixatives are evaluated (organism morphology after permanent staining). Additional fixatives prepared with non mercuric chloride-based compounds are continuing to be developed and tested.

<sup>2</sup>This modification uses a copper sulfate base rather than mercuric chloride.

<sup>3</sup>This modification uses a zinc base rather than mercuric chloride and apparently works well with both trichrome and iron-hematoxylin stains.

<sup>4</sup>These modifications use a combination of ingredients (including zinc), but are prepared from proprietary formulas. The aim is to provide a "universal fixative" that can be used for the fecal concentration, permanent stained smear, and available immunoassays for *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica* (or the *Entamoeba histolytica*/*E. dispar* group). Fecal immunoassays for the *Entamoeba histolytica*/*E. dispar* group or *Entamoeba histolytica* (true pathogen) require fresh or frozen specimens; testing can also be performed from stool submitted in Cary-Blair transport medium. Examples of two "Universal fixatives" are: SAF (contains formalin), TOTAL-FIX (no formalin).

EIA, enzyme immunoassay; cartridge, rapid membrane flow method; FA, fluorescent antibody; MIF, merthiolate-iodine-formalin; ND, no data; PVA, polyvinyl alcohol; SAF, sodium acetate-acetic acid-formalin

### **COMMENTARY:**

The most common collection option (original public health approach) is a two vial system: one vial of 5% or 10% formalin and one vial of fixative containing the plastic adhesive, polyvinyl alcohol (PVA). The formalin vial is used for the concentration and fecal immunoassays, while the PVA vial is used for the permanent stained smear. Regulations for formalin (see below) were originally developed for industry, not the clinical laboratory where amounts of formalin tend to be quite low.

### **Formalin**

Formalin has been used for many years as an all-purpose fixative that is appropriate for helminth eggs and larvae and for protozoan cysts, oocysts, and spores. Two concentrations are commonly used: 5%, which is recommended for preservation of protozoan cysts, and 10%, which is recommended for helminth eggs and larvae. Although 5% is often recommended for all-purpose use, most commercial manufacturers provide 10%, which is more likely to kill all helminth eggs. To help maintain organism morphology, the formalin can be buffered with sodium phosphate buffers, i.e., neutral formalin. Selection of specific formalin formulations is at the user's discretion. *Aqueous formalin will permit the examination of the specimen as a wet mount only, a technique much less accurate than a permanent stained smear for the identification of intestinal protozoa.* However, the fecal immunoassays for *Giardia lamblia* and *Cryptosporidium* spp. can be performed from the aqueous formalin vial. Fecal immunoassays for the *Entamoeba histolytica*/*E. dispar* group and *Entamoeba histolytica* are limited to fresh or frozen fecal specimens. After centrifugation, special stains for the coccidia (modified acid-fast stains) and the microsporidia (modified trichrome stains) can be performed from the concentrate sediment obtained from formalin-preserved stool material. Use of the sediment provides a more sensitive test.

### **Current OSHA Regulations on the Use of Formaldehyde**

Formaldehyde has been in use for over a century as a disinfectant and preservative, it is also found in a number of industrial products. There is disagreement about the carcinogenic potential of lower levels of exposure and epidemiologic studies of the effects of formaldehyde exposure among humans have given inconsistent results. Studies of industry workers with known exposure to formaldehyde report little evidence of increased cancer risk. It also appears that persons with asthma respond no differently than healthy individuals following exposure to concentrations of formaldehyde up to 3.0 ppm.

OSHA requires all workers to be protected from dangerous levels of vapors and dust. Formaldehyde vapor is the most likely air contaminant to exceed the regulatory threshold in a laboratory, particularly in anatomic pathology. Current OSHA regulations require vapor levels not to exceed 0.75 ppm (measured as a time-weighted average [TWA]) and 2.0 ppm (measured as a 15-min short-term exposure). *OSHA requires monitoring for formaldehyde vapor wherever formaldehyde is used in the work place. The laboratory must have evidence at the time of inspection that formaldehyde vapor levels have been measured, and both 8-h and 15-min exposure must have been determined.*

If each measurement is below the permissible exposure limit and the 8-h measurement is below 0.5 ppm, no further monitoring is required as long as laboratory procedures remain constant. If the 0.5-ppm 8-h TWA or the 2.0-ppm 15-min level is exceeded, monitoring must be repeated semiannually. If either the 0.75-ppm 8-h TWA or the 2.0-ppm 15-min level is exceeded (**VERY UNLIKELY in a routine microbiology laboratory setting**), employees must be required to wear respirators. Accidental skin contact with aqueous formaldehyde must be prevented with the use of proper clothing and equipment (gloves, laboratory coats).

The amendments of 1992 add medical removal protection provisions to supplement the existing medical surveillance requirements for employees suffering significant eye, nose, or throat irritation and for those experiencing dermal irritation or sensitization from occupational exposure to formaldehyde. In addition, these amendments establish specific hazard-labeling requirements for all forms of formaldehyde, including mixtures and solutions composed of at least 0.1% formaldehyde in excess of 0.1 ppm. Additional hazard labeling, including a warning label that formaldehyde presents a potential cancer hazard, is required where formaldehyde levels, under reasonably foreseeable conditions of use, may potentially exceed 0.5 ppm. The final amendments also provide for annual training of all employees exposed to formaldehyde at levels of 0.1 ppm or higher.

**Note** The use of monitoring badges may not be a sensitive enough method to correctly measure the 15-min exposure level. Contact the Occupational Health and Safety Office within your institution for monitoring options. Usually, the accepted method involves monitoring air flow in the specific area(s) within the laboratory where formaldehyde vapors are found.

### **PVA**

PVA is a plastic resin that is normally incorporated into Schaudinn's fixative. The PVA powder is NOT a fixative, but serves as an adhesive for the stool material; i.e., when the stool-PVA-fixative mixture is spread onto the glass slide, it adheres because of the PVA component. Fixation is still accomplished by the Schaudinn's fluid (or other fixative) itself. Perhaps the greatest advantage of the use of PVA is the fact that a permanent stained smear can be prepared. Although some laboratories may perform a fecal concentration from a PVA-preserved specimen, some parasites will not concentrate well, nor will some exhibit the typical morphology that would be seen in concentration sediment from a formalin-based fixative. PVA fixative solution is highly recommended as a means of preserving cysts and trophozoites for later examination. The use of PVA fixative also permits specimens to be shipped (by regular mail service) from any location in the world to a laboratory for subsequent examination. PVA fixative is particularly useful for liquid specimens and should be used in the ratio of 3 parts PVA to 1 part fecal specimen.

**NOTE:** Very detailed information on all fixative options can be found in:  
Garcia, L.S. Diagnostic Medical Parasitology, 5<sup>th</sup> ed., ASM Press, Washington D.C., 2007.