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Evaluation of Intestinal Parasite Morphology in Polyvinyl Alcohol Preservative: Comparison of Copper Sulfate and Mercuric Chloride Bases for Use in Schaudinn Fixative

LYNNE S. GARCIA,* ROBYN Y. SHIMIZU, THOMAS C. BREWER, AND DAVID A. BRUCKNER

Department of Pathology, Clinical Microbiology, University of California at Los Angeles Medical Center, Los Angeles, California 90024

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As a result of disposal problems inherent in the use of mercury compounds, many laboratories have considered using copper sulfate as a substitute for mercuric chloride in polyvinyl alcohol (PVA) preservative. The primary use for PVA-preserved specimens is the permanent stained smear, the most important technique for the identification of intestinal protozoa. A comparison of organism recovery and morphology was undertaken with PVA containing either copper sulfate or mercuric chloride base. Paired fecal specimens (417 pairs) were collected and examined with the Formalin-ether concentration and Trichrome stain techniques. Numbers of organisms recovered and helminth egg and protozoan morphology were assessed from the concentration sediment. Morphology, clarity of nuclear and cytoplasmic detail, overall color differences, and the ease or difficulty in detecting organisms in fecal debris were assessed from the permanent stained smear. No significant differences were found in the numbers and morphology of organisms seen in the concentration sediment. However, when the trichrome stain was used, the overall morphology of the intestinal protozoa preserved in PVA with copper sulfate was not equal to that seen with PVA with mercuric chloride. We do not recommend switching from mercuric chloride base to copper sulfate base unless that is the only option available for the preparation of permanent stained smears.

For many years Schaudinn and polyvinyl alcohol (PVA) fixatives with a mercuric chloride ($HgCl_2$) base have been used to preserve stool specimens for the recovery and identification of intestinal parasites (1, 2). The concentration technique for either 10% Formalin- or PVA-preserved specimens has been used for the recovery of helminth eggs and larvae and protozoan cysts. The permanent stained smear prepared from Schaudinn or PVA fixative is used primarily for the identification of intestinal protozoa and is considered to be the most important technique for this purpose (3, 5, 6, 9). During the past few years the question of mercury disposal has been raised by clinical laboratories; many facilities do not have the ability to dispose of small quantities of materials contaminated with mercury compounds. The use of Schaudinn fluid prepared with some compound other than $HgCl_2$ would be a definite advantage for laboratories performing ova and parasite examinations on stools. Since several manufacturers offer PVA fixative with the Schaudinn component prepared with copper sulfate ($CuSO_4$), a clinical study was undertaken to compare organism detection and morphology with both types of PVA fixative, one with $HgCl_2$ and the other with $CuSO_4$ (7).

MATERIALS AND METHODS

Fecal specimens were collected in 417 paired vials, one containing PVA with $HgCl_2$ and the other containing PVA with $CuSO_4$. Formalin-ether concentrations were performed on each vial with identical amounts of fecal-preservative mix for each. Concentrates and permanent stained smears were coded so that the preservative base was not revealed until after the study was completed. Permanent stained smears were prepared from each vial with the Trichrome procedure (4, 8). Both concentrates and smears were read, and the results were recorded by technologists other than those preparing the concentrates and smears. Numbers of organisms recovered and helminth egg and protozoan morphology were assessed from the concentration sediment. The clarity of nuclear and cytoplasmic detail, overall staining differences, and the ability to detect organisms in fecal debris were assessed from the permanent stained smear for both trophozoite and cyst stages by four experienced technologists. Data from the paired vials were then compared for both helminth eggs and protozoa.

TABLE 1. Comparative morphology of pathogenic protozoa on Trichrome permanent stained smears with mercuric chloride or copper sulfate in PVA fixative

Organism	No. (%) of pathogenic protozoa for which:		
	Equal results were obtained with $HgCl_2$ and $CuSO_4$	Better results were obtained with $HgCl_2$	Better results were obtained with $CuSO_4$
Entamoeba histolytica			
Trophozoites	2 (15)	8 (62)	3 (23)
Cysts	4 (33)	6 (50)	2 (17)
Giardia lambia			
Trophozoites	0	2 (100)	0
Cysts	31 (61)	16 (31)	4 (8)
Dientamoeba fragilis trophozoites	6 (40)	6 (40)	3 (20)

RESULTS

There were instances in which the comparative morphology of the pathogenic protozoa was equal, depending upon the species, although the best overall nuclear and cytoplasmic detail and clarity were seen with $HgCl_2$, particularly with the trophozoite stages (Table 1). The percentages also varied from species to species for the nonpathogenic protozoa (Table 2). Although $CuSO_4$ produced better morphology in some instances, $HgCl_2$ produced much better overall morphology. No differences in numbers of organisms detected on the permanent stained smears were noted. The majority of the smears prepared from both fixatives showed no significant differences in color which would influence the ability to recognize the organism (Table 3). If there were differences, color obtained from $HgCl_2$ and subsequent trichrome staining was better than that obtained with $CuSO_4$. The number of specimens positive for helminth eggs and larvae was small (Table 4); however, there appeared to be no significant differences in numbers recovered and morphology per paired specimens upon examination of the concentration sediment.

TABLE 2. Comparative morphology of nonpathogenic protozoa on Trichrome permanent stained smears with mercuric chloride or copper sulfate in PVA fixative

Organism	No. (%) of nonpathogenic protozoa for which:		
	Equal results were obtained with $HgCl_2$ and $CuSO_4$	Better results were obtained with $HgCl_2$	Better results were obtained with $CuSO_4$
Entamoeba hartmanni			
Trophozoites	2 (18)	8 (73)	1 (9)
Cysts	5 (38)	7 (54)	1 (8)
Entamoeba coli			
Trophozoites	5 (29)	7 (42)	5 (29)
Cysts	39 (50)	29 (38)	9 (12)
Endolimax nana			
Trophozoites	16 (36)	19 (42)	10 (22)
Cysts	33 (40)	44 (53)	6 (7)
Iodamoeba bütschlii			
Trophozoites	4 (57)	3 (43)	0
Cysts	7 (64)	4 (36)	0
Trichomonas hominis trophozoites	1 (100)	0	0
Enteromonas hominis			
Trophozoites	0	0	0
Cysts	1 (100)	0	0

TABLE 3. Comparative color differences among Trichrome-stained intestinal protozoa fixed with either mercuric chloride or copper sulfate in PVA preservative

Organism	No. (%) of protozoa for which[a]:	
	There were no differences	Better results were obtained with $HgCl_2$
Entamoeba histolytica	25 (100)	0
Giardia lamblia	47 (89)	6 (11)
Dientamoeba fragilis	15 (100)	0
Entamoeba hartmanni	23 (96)	1 (4)
Entamoeba coli	79 (84)	15 (16)
Endolimax nana	108 (84)	20 (16)
Iodamoeba bütschlii	16 (89)	2 (11)
Trichomonas hominis	1 (100)	0
Enteromonas hominis	1 (100)	0

[a] Better results were never obtained with $CuSO_4$.

TABLE 4. Morphology of helminth eggs and larvae in concentration sediment obtained from material fixed with PVA with either mercuric chloride or copper sulfate

Organism	No. (%) of helminth eggs and larvae for which[a]:	
	Equal results were obtained with $HgCl_2$ and $CuSO_4$	Better results were obtained with $CuSO_4$
Ascaris lumbricoides	19 (100)	0
Trichuris trichiura	25 (100)	0
Hookworm	4 (100)	0
Strongyloides stercoralis	0	1 (100)
Taenia sp.	3 (100)	0
Hymenolepis nana	4 (100)	0

[a] Better results were never obtained with $HgCl_2$.

DISCUSSION

Although $HgCl_2$ has traditionally been used in the preparation of PVA, several other compounds have been tried as substitutes because of potential problems with mercury disposal. This particular study was designed to test one of the possible substitutes, $CuSO_4$, and to determine its effectiveness compared to that of $HgCl_2$. When concentration sediments were examined, the overall differences in both recovery and morphology of helminth eggs and larvae and protozoa were minimal and probably not clinically significant in terms of organism identification. When permanent stained smears were examined, there were some overall color differences; these differences were minimal, and in less than 20% of the paired smears was the color significant in influencing the ability to detect morphological differences among the intestinal protozoa. Stained smears prepared from $CuSO_4$-fixed material were green-blue in color, and $HgCl_2$ smears were more uniformly blue, with better differential colors (purple, red, pink); however, organisms were detectable on both types of smears.

The actual nuclear and cytoplasmic detail and clarity of the intestinal protozoa were different when the paired smears were compared. Smears prepared from PVA with $HgCl_2$ were consistently better quality, with clear, well-defined details. Those smears prepared from PVA with $CuSO_4$ did not provide the same clarity and definition of morphological characteristics necessary for identification. The overall organism fixation appeared to be inadequate (fuzzy detail, shrinkage, poorly defined nuclear detail), and final protozoan identification was often difficult at best.

The recovery and identification of intestinal protozoa are frequently dependent upon the use of the permanent stained smear, and it is important that this technique be performed to maximize identification capabilities. The ability to identify the organisms after staining is dependent upon obtaining the best possible fixation of the specimen as soon as possible after passage (10). Because the overall morphology of the intestinal protozoa was not as good when PVA with $CuSO_4$ was used as when PVA with $HgCl_2$ was used, we do not recommend switching from $HgCl_2$ to $CuSO_4$ unless one cannot use fixatives with a mercury base. The use of the $CuSO_4$ base provides a permanent stained smear which is not equal in quality to that provided by use of an $HgCl_2$ base, but any permanent stain will certainly increase the chances for protozoan recovery and identification over that with concentration sediment alone.

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