Protozoans in Stools Unpreserved and Preserved In PVA-Fixative

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In an earlier report (*1*), we demonstrated the effectiveness of polyvinyl alcohol (PVA) fixative in preserving the trophozoites of the intestinal amebas and recommended that it be incorporated in a two-bottle outfit for the collection of stool specimens whenever it was not possible to have them examined immediately. This method of collection is particularly applicable to public health laboratories which generally receive specimens for diagnosis through the mail. The present study compares the relative effectiveness of the PVA-fixative technique and other procedures in detecting intestinal protozoans in feces.

Materials and Methods

Arrangements were made with the Grady Memorial Hospital in Atlanta whereby stool specimens were collected and sent to the Communicable Disease Center laboratory of the Public Health Service in Atlanta for examination. Part of each normally passed, fresh stool was immediately preserved in PVA-fixative, and a part was left unpreserved. All specimens were over 4 hours old when they reached the laboratory.

Patients submitting stools included new hospital admissions and individuals suspected of having amebic or other intestinal infections. Five hundred specimens were submitted from approximately 270 patients. Since the specimens were generally identified only by the patient's last name, it was not possible to tell in every instance whether the patient was a repeat case or a new one. For these reasons, the percentages of the parasites found in this study do not indicate infection rates of a population. They represent only what was found in 500 separate stool specimens examined by various techniques.

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Examination

The unpreserved portions were examined by direct wet mounts (saline and iodine), modified zinc sulfate concentrations, and hematoxylin-stained direct smears, the methods for which are described in techniques 1, 2, and 3 below. The PVA-preserved portions were examined by hematoxylin-stains of PVA films, as explained in technique 4.

Technique 1. A fleck of feces was mixed with a drop of saline and covered with a 22 mm. square cover slip. A similar preparation was made using an iodine solution. The entire saline mount was carefully examined. The iodine mount was used to assist in identifying organisms which were found in the saline preparation.

Technique 2. Approximately 1 gm. of feces was mixed with tapwater in a 14 by 85 mm. test tube. The test tube was centrifuged at 2,000 r. p. m. for 1 minute. The supernatant was poured off, and the tube was refilled not quite to the top with zinc sulfate solution (specific gravity 1.18). After a second centrifugation at 2,000 r. p. m. for 1 minute, the tube was placed on a rack, and sufficient zinc sulfate solution was added with a dropper to raise the meniscus above the top of the tube. A 22 mm. square cover slip was carefully superimposed on the tube and allowed to remain undisturbed for 5 to 10 minutes. At the end of that time, the cover slip was removed, lowered onto a drop of iodine solution on a slide, and examined.

Technique 3. Flecks of feces were spread in thin films on two 75 by 25 mm. slides, which were immediately immersed in Schaudinn's fixative. One slide was then stained by the Tompkins-Miller rapid hematoxylin technique (*2*). When the results of this method were not criti-

cal enough, the Heidenhain iron-hematoxylin procedure was used to stain the duplicate slide.

Technique 4. A drop of the sediment from each portion preserved in PVA-fixative was pipetted onto two 75 by 25 mm. slides, spread over an area approximately 25 mm. square, and allowed to dry overnight in a 37° C. incubator. The two dried films were stained in the same manner as described under technique 3.

The various preparations were examined for as long as was necessary to satisfy the examiner of the diagnosis. Inasmuch as the reports of these examinations were used by the hospital in the management of the patient, some checking back and forth concerning the results of the various techniques occurred in order to confirm or deny the presence of a given organism in a patient.

Results and Discussion

The hematoxylin-stained films of the portions preserved in PVA-fixative revealed more positives than did any one of the techniques performed on the unpreserved portions of the same specimens—146 as compared to 92 for the next most efficient procedure (table 1). It should be noted that no infections with *Dientamoeba fragilis* would have been found under the conditions of this survey if PVA-fixative had not been used. In the past, this species has been considered extremely rare. The use of a preservative like PVA-fixative should make possible a truer picture of the incidence of this form. Such information would be valuable in view of the possible pathogenic character of this organism (*3*).

Trophozoites. The greater efficiency of the PVA-fixative method is primarily due to its ability to preserve the trophozoites of the intestinal protozoans which in an unpreserved stool ordinarily deteriorate beyond recognition within a few hours. In this study, the PVA-fixed portion revealed 100 percent (124 of 124) of all the detected infections with trophozoites, whereas the most efficient of the procedures used on the unpreserved portions revealed only 14 percent (17 of 124) of the trophozoites (table 1).

This effectiveness of PVA-fixative in preserv-

Table 1. Number of infections with intestinal protozoans, trophozoites, and cysts (identified or not) found in 500 stool specimens examined by 4 techniques

Organisms	Type of specimen and techniques used				
	Unpreserved			Preserved in PVA-fixative	All four techniques combined
	(1) Direct wet mount	(2) Modified zinc sulfate concentration	(3) Hematoxylin-stained direct smear	(4) Hematoxylin-stained PVA film	
Endamoeba histolytica	10	17	19	28	38
Endamoeba coli	23	30	19	25	39
Endolimax nana	26	35	40	67	71
Iodamoeba butschlii	4	4	3	6	7
Dientamoeba fragilis	0	0	0	7	7
Giardia lamblia	6	6	9	9	11
Chilomastix mesnili	0	0	1	2	2
Small flagellates	0	0	0	2	2
Total	69	92	91	146	178
Unidentified organisms	15	9	5	16	15
Positive stool specimens	68	73	77	113	132
Trophozoites	13	0	17	124	124
Cysts	73	97	87	64	123

Table 2. Number of infections with trophozoites and cysts (identified or not) found in 500 stool specimens listed according to consistency of specimen

Type of stool	Number of specimens	Trophozoites only (a)	Cysts only (b)	Trophozoites with or without cysts (c)	Total infections (b plus c)
		Number	*Number*	*Number*	*Number*
Formed	350	36	56	78	134
Soft	150	36	18	48	66
Total	500	72	74	126	[1] 200

[1] 7 of these were unidentified cysts or trophozoites of species that were considered to be identified on the basis of the combined findings of all techniques.

ing trophozoites would not in itself assure an increase in the number of infections detected unless trophozoites were present in the normally passed stool much more frequently than is generally believed to be so. In this study (see table 2), it was found that of 200 infections (identified or not) found by all 4 techniques, 126 (63 percent) contained trophozoites with or without cysts; the number of infections containing trophozoites only was 72 (36 percent). It is therefore apparent that any method that makes possible the detection of trophozoites will materially increase the number of positives which may be found.

It has been believed generally that amebic trophozoites are to be found primarily in soft stools, and infrequently in formed stools. (The term "soft" is used here to include all categories of stool which cannot be described as definitely formed.) If that were true, then the use of PVA-fixative could be restricted to soft specimens. The present observations do not appear to confirm this point of view. Of 134 infections found in formed stools, 78 (59 percent) showed trophozoites either alone or with cysts; and 36 (27 percent) showed trophozoites only (table 2). Thus, it would seem advisable to preserve even formed stools in PVA-fixative; otherwise, a sizable number of infections are likely to go undetected.

Cysts. The PVA-fixative method is relatively inefficient with respect to the identification of amebic cysts. Any one of the methods used on the unpreserved portions revealed a greater number of cysts than did the stained smears of the PVA-fixed portions (table 1). The question therefore arises as to whether the use of PVA-fixative would increase the number of positives above that which could be found by using all the three older techniques combined.

The hematoxylin-stained PVA film by itself revealed more infections than did all the techniques performed on the unpreserved portions put together (table 3). This was due primarily to the greater number of *Endolimax nana* infections which were detected in the PVA films. There was no significant increase in the number of *Endamoeba histolytica* infections, and there was a distinct decrease in the number of *Endamoeba coli* infections found.

Combined Techniques. Since no one method in this investigation was equally efficient for both trophozoites and cysts, it is obvious that the laboratory which is interested in recovering the greatest number of positives must plan to use at least 2 techniques to insure the finding of both stages.

Of the 4 techniques used here, the method of choice for trophozoites is, as has been shown, PVA-fixative. The number of infections obtained by combining the findings of the PVA-fixative method with those of each of the other methods used is shown in table 3. No combination of 2 techniques recovered as many positives as did all 4 techniques together. This reflects the experience of most diagnostic laboratories: The more a stool specimen is examined by various methods, the greater is the likelihood of finding the rare individual protozoan which may be present.

From a practical standpoint, however, the combination of the PVA-fixative and the zinc

Table 3. Number of identified infections found by various combinations of techniques

Combination of techniques	*Endamoeba histolytica*		*Endamoeba coli*		*Endolimax nana*		All species	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Unpreserved portions (techniques 1, 2, and 3)	27	71	32	82	44	62	117	66
Hematoxylin-stained PVA film	28	74	25	64	67	94	146	82
PVA-fixative plus direct wet mount	28	74	33	85	68	96	156	88
PVA-fixative plus modified zinc sulfate concentration	33	87	39	100	70	99	169	95
PVA-fixative plus hematoxylin-stained direct smear	33	87	29	74	70	99	160	90
All 4 techniques	38	100	39	100	71	100	178	100

sulfate methods would appear to be the most efficient combination of techniques. With these 2 methods it was possible to find 95 percent of the identified infections found by all 4 methods (169 of 178). The other 2 combinations—PVA-fixative plus hematoxylin-stained direct smears and PVA-fixative plus direct wet mounts—revealed 90 and 88 percent, respectively, of the total infections identified.

Unidentified Organisms. Of 16 cases where organisms were seen but were not specifically identified in the PVA-fixed portions (table 1), 12 (75 percent) were trophozoites. In most cases, these were *Endamoeba* organisms which could not be diagnosed definitely as either *histolytica* or *coli*. In our experience it has been impossible to identify specifically a proportion of *Endamoeba* trophozoites in stained preparations on the basis of the classic descriptions of the two intestinal species.

Since, in this study, the unpreserved specimens were several hours old when examined, the organisms not identified by the other techniques were mostly rare distorted cysts or degenerated trophozoites. When the resources of all 4 techniques were used, the number of organisms not identified was 15 compared to 178 identified, or 8 percent of all organisms seen.

Summary

Five hundred normally passed stool specimens were divided into two portions immediately after passage. One portion was left unpreserved; the other was preserved in PVA-fixative. The unpreserved portions were examined by direct wet mounts, zinc sulfate concentrations, and hematoxylin-stained direct smears. The preserved portions were examined by hematoxylin stains of PVA films. All examinations were performed no sooner than 4 hours after the stool was passed.

The PVA-fixative portions revealed more infections with protozoans than did all 3 other techniques combined, mainly as a result of the preservation of trophozoites. Trophozoites were found in 63 percent of all infections detected and in 59 percent of the infections found in formed stools. This suggests that it is advisable to preserve both formed and soft stools in PVA-fixative. The combination of the PVA-fixative method (for trophozoites) and the zinc sulfate method (for cysts) demonstrated more infections than did any other combination of 2 techniques used in this study.

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