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Examination of Preserved Stool Specimens for Parasites: Lack of Value of the Direct Wet Mount

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To review the appropriateness of standard reference procedures for diagnostic parasitology, we examined 2,206 stool specimens in our laboratory by direct wet mounting with saline and iodine, by saline and iodine wet mounting from Formalin-ethyl acetate concentrates, and by permanent staining with Wheatley's modified trichrome method (W. B. Wheatley, Am. J. Clin. Pathol. 21:990–991, 1951). Parasites were detected in 98 stool specimens (4.4%). In all but three specimens, direct wet mounting with saline and iodine contributed little significant information to the result yet consumed substantial technical time. We recommend that with preserved feces a direct examination not be performed but that examination of both a concentrate and a permanent stain be routine.

Clinical microbiology texts differ on the procedures recommended for the examination of stools, particularly preserved feces, for parasites. Two major diagnostic parasitology texts recommend that direct wet mounts (DWM) be examined when formalinized stool samples are received (4, 7); a major clinical microbiology text does not specify if DWM should be prepared (1). Many laboratories no longer examine DWM from formalinized stools; however, we could find no data in the literature to support this omission. Traditionally, reasons for the use of DWM include the following: (i) it is a fast, simple procedure and provides a quick answer when positive (3); (ii) it provides an estimate of the parasitic burden (3); (iii) it can be used with unpreserved specimens to detect the characteristic motility of trophozoites (7); and (iv) it can be used as a safeguard, as some protozoa may at times not concentrate properly because of unknown factors (8).

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A total of 2,206 stool specimens were received in our laboratory for routine parasitologic examination. Instructions for collection, detailed in our hospital's laboratory manual, include directions for preservation of the specimens as soon as possible after collection. All of the specimens included in this study were received in a two-vial kit (ParaPak; Meridian Scientific, Cincinnati, Ohio). The kit contains one vial of 10% Formalin and one vial of polyvinyl alcohol preservative.

All specimens were processed by three techniques. (i) Direct examination was accomplished with the Formalin-preserved portion of the specimen by preparing two wet mounts before processing the specimen further. One wet mount was prepared with physiologic saline, and the other was prepared with Lugol's iodine solution diluted 1:5. (ii) The Formalin-ethyl acetate (FEA) concentration method was performed with the remaining formalinized specimen as described by Young et al. (10). (iii) The polyvinyl alcohol-preserved portion was used to prepare two smears, one of which was stained by Wheatley's modified trichrome method (9). The second smear remained unstained as a backup. Wet mounts were examined by systematically scanning the entire cover slip (7). Trichrome stains were examined at a 1,000× magnification under oil immersion for approximately 10 to 15 min. All examinations were performed by a certified medical technologist assigned to our parasitology section.

Of 2,206 specimens examined between January 1981 and August 1983, a total of 98 (4.4%) were found to be positive for one or more parasites; this positive rate is comparable to that found in other hospitals in our area. Of the 98 positives, 92 were in sufficient quantity to allow for the comparison of processing by each of the three procedures. Trichrome staining exclusively revealed parasites in 19 (20.6%) specimens. FEA concentration exclusively revealed parasites in 14 (15.2%) specimens. Direct examination exclusively revealed parasites in three (3.3%) specimens. A total of 112 parasites, representing 15 species, were recovered from the 98 positive specimens (Table 1).

A total of 26 parasites, all protozoa, were found in the 19 specimens positive by trichrome staining only. Of these parasites, 12 were in the cyst (C) stage and 14 were in the trophozoite (T) stage. The 26 protozoa were divided as follows: 8 *Giardia lamblia* (7 C and 1 T), 8 *Endolimax nana* (2 C and 6 T), 4 *Entamoeba coli* (1 C and 3 T), 2 *Chilomastix mesnili* (2 C and T), 3 *Entamoeba histolytica* (1 C and 2 T), and 1 *Dientamoeba fragilis*.

A total of 14 parasites, 8 helminths and 6 protozoa, were recovered from the 14 specimens positive by FEA concentration only. Parasites found in this group were as follows: five *Strongyloides stercoralis*, three *G. lamblia*, three *E. coli*, one *Taenia* species, one *Clonorchis* or *Opistorchis* species, and one *Trichuris trichiura*.

One parasite was recovered from the three specimens positive by direct examination only; in all three, the parasite found was the egg stage of *Enterobius vermicularis*.

These results were evaluated to determine the contribution of each procedure to the final result and to either justify the "full workup" given to each specimen or trim the technical time required for examination without sacrificing quality. It should be emphasized that all the specimens in this study were received in preservatives, and therefore our

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TABLE 1. Parasite recovery by various methods

Parasite	No. of parasites recovered by:			Total no. of parasites recovered
	Direct wet mounting	FEA concentration only	Trichrome staining	
G. lamblia	0	3	8	42
E. coli	0	3	4	22
E. nana	0	0	8	20
S. stercoralis	0	5	0	6
E. histolytica	0	0	3	3
Iodamoeba butschlii	0	0	0	3
E. vermicularis	3	0	0	3
T. trichiura	0	1	0	3
Ascaris lumbricoides	0	0	0	2
Hookworm species	0	0	0	2
C. mesnili	0	0	2	2
Taenia sp.	0	1	0	1
D. fragilis	0	0	1	1
Clonorchis or *Opistorchis* sp.	0	1	0	1
Entamoeba sp.	0	0	0	1

data may not be applicable to fresh, unpreserved feces. Markell and Quinn (6) demonstrated that the preservation of fecal specimens immediately after passage yielded an increased recovery of parasites. In addition, a laboratory using a different method for concentrating feces, such as the zinc sulfate centrifugal flotation method, may also find our data not applicable, as recovery by that method may be different for various parasites.

Trichrome staining exclusively revealed parasites in 20.6% (19/92) of positive specimens. Because we receive, almost exclusively, preserved stools, we rarely have any information as to the consistency of the specimen and consequently cannot reserve trichrome staining for diarrheic stools only. It has been recommended, however, that permanent staining be performed on all stools, regardless of consistency (1, 4, 7, 8). In our setting, trichrome staining is essential. Parasites detected exclusively in the trichrome stain were always protozoans and were about evenly divided between cysts and trophozoites (13 cysts and 15 trophozoites). The exclusive detection by trichrome staining of both cysts and trophozoites indicates that there may often be cysts that go undetected in wet mounts, probably because of both scarcity and small size. The phenomenon of protozoan cysts that do not concentrate normally cannot be ignored and may account for cysts not being detected in wet mounts of concentrates; this effect has been reported with the Formalin-ether concentration method (8), but we have also seen it occur rarely with the FEA concentration method. Interestingly, the three specimens positive for *E. histolytica* were found only by trichrome staining. Also, a laboratory not performing permanent stains will rarely detect *D. fragilis*, as this organism has no cyst stage (4).

Examination of wet mounts of concentrates allowed the detection of parasites in 15.2% (14 of 92) of the positive specimens. This was the only positive procedure for these specimens, and the parasites recovered were four helminth species in eight specimens and two protozoan species in six specimens. Although we have occasionally recovered protozoan trophozoites from concentrates, none was observed in these specimens.

Direct wet mounting exclusively revealed parasites in three specimens. These three specimens were positive for eggs of the pinworm, *E. vermicularis*. Essentially then, examination of DWM provided little additional information beyond that available from the concentrate and the trichrome stain in 96.7% (89 of 92) of positive specimens and in 99.9% (2,203 of 2,206) of the total number of specimens. Furthermore, it is well recognized that only about 5% of patients infected with pinworms will have eggs demonstrated in their stools (2). Because of this, a stool sample is not the recommended specimen to use in searching for pinworms; the cellophane tape preparation is preferred.

We conclude that, in our setting, examination of DWM provides little added advantage while consuming substantial technical time. Currently, the College of American Pathologists Workload Recording System allows a time value of 7 min per cover slip for microscopic examination of wet mounts (5). Estimating from our current specimen work load, we save approximately 23 h per month by eliminating DWM. Of course, no technologist works at 100% efficiency, so that a full 23 h translates into more than that in real personnel time.

In summary, with formalinized specimens, DWM are of minimal value, provided that FEA concentration is performed on the specimen and that a permanent stain is prepared from a polyvinyl alcohol-preserved portion of the stool. In this study, 20% of positives would have been missed had trichrome staining not been routine.

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