Evaluation of Direct Wet Mount Parasitological Examination of Preserved Fecal Specimens

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A retrospective study covering a 12-year period was performed to determine the contribution of the direct wet mount microscopic examination to the identification of intestinal parasites in preserved fecal material. It was determined that each of 898 specimens contained at least one parasite when processed by the direct wet mount, concentration, and trichrome staining procedures. Of these procedures, the direct wet mount examination was solely responsible for the identification of 45 (2.9%) of 1,581 parasites identified. This is in contrast to the 15.1 and 12.5% which were found exclusively by the concentration and trichrome methods, respectively. These percentages may vary, depending on the prevalence of parasites and the stages present in the stool specimen.

For a number of years, the Centers for Disease Control (5) has recommended that the routine parasitological examination of a fecal specimen consist of a direct macroscopic examination, a direct wet mount examination in both saline and iodine, a concentration examination (preferably one incorporating a defatting agent and centrifugation), and a permanently stained smear. In contrast, the Manual of Clinical Microbiology published by the American Society for Microbiology (6) is more conservative in that the authors find that the direct wet mount examination is essential only for the examination of soft-to-watery fecal specimens. The direct wet mount examination of these types of fresh specimens has a unique advantage not shared with the concentration or trichrome procedure, i.e., the direct wet mount can detect the motile trophozoite stage of the protozoan species. However, timely processing, within 1 h of passage (1, 4, 5, 7), of a fresh specimen is not always possible in a busy clinical laboratory or in a laboratory which processes only mailed specimens. In addition, a definitive identification of trophozoites is usually not possible in this type of preparation (3, 5).

Recently, the value of the direct wet mount examination was reported in the literature (2, 8). Estevez and Levine (2) reported on 92 preserved fecal specimens which were processed by the direct, concentration, and trichrome procedures. They found that the direct wet mount was exclusively responsible for detecting only 3 of the 112 parasites identified. Watson et al. (8) retrospectively compared the results of 147 fecal specimens, most of which they indicated were unprocessed, processed by the direct and concentration procedures. They did not find any parasites which were identified only by the direct wet mount examination.

Because of the disparate recommendations appearing in the two authoritative manuals regarding the inclusion of the direct wet mount examination in the routine diagnostic parasitology regimen and the limited number of specimens examined in the two studies cited above, a more extensive study was performed. The study design recognized and accounted for certain important factors which can greatly influence the overall recovery of intestinal parasites, i.e., the consistency of the specimen (watery versus formed), the use of preservatives and their ratio in the specimen, the procedure itself, and the diligence and ability of the person performing the examination.

All our parasitology laboratory reports for a 12-year period were reviewed. Only those reports on fecal specimens meeting the following criteria were included in this study: (i) received in two-vial kits, prepared and provided by this laboratory, consisting of one vial containing polyvinyl alcohol fixative and a second vial containing 5% Formalin (1, 5, 7) along with detailed instructions for use by the patient; (ii) processed by the direct wet mount microscopic procedure, a concentration procedure, and the trichrome staining procedure; and (iii) positive for intestinal parasites by at least one of the above methods. A total of 898 specimens which were submitted to the State Laboratory (Lionville, Pa.) by State Health Centers, physicians, and other health care providers met the criteria for inclusion in this study.

All specimens were processed by three techniques. (i) The direct wet mount examination was performed on the Formalin-preserved sample and consisted of both saline and iodine (Dobell) preparations as described elsewhere (5). The entire Vaspar-sealed preparations (25 by 25 mm) were systematically examined at a magnification of ×100 by using overlapping microscopic fields. When suspicious objects or organisms were seen, confirmation was made by using a magnification of ×430 for helminth eggs and larvae and a magnification of ×970 for protozoan cysts and trophozoites. (ii) Because of the lengthy period covered in this study, 743 specimens were concentrated by the Formalin-ether technique (5) and 155 were concentrated by the Formalin-ethyl acetate technique (9). For both of these concentration procedures, the Formalin-preserved specimens were filtered through a single layer of wet gauze into a 15-ml conical centrifuge tube. Examination of the sediment was identical to that described for the direct wet mounts. (iii) The slides of the polyvinyl alcohol-preserved feces were prepared and stained by the trichrome method (5), and the entire smear (25 by 40 mm) was systematically examined at a magnification of ×970. All specimens included in this study were processed and examined by the same parasitologist.

The data for the identified parasites generated in this retrospective study were managed in two ways: by the methods by which they were identified and by the method which exclusively permitted their identification. A total of 486 helminths and 1,095 protozoans were

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identified (Table 1) in the 898 specimens included in this study. All of the helminths, except Strongyloides stercoralis, which was identified by the recognition of the rhabditiform larval stage, were identified by the recognition of the egg stage. The protozoans were identified by the recognition of the cyst (C) and/or the trophozoite (T) stage, which numbered 878 and 457, respectively.

The direct wet mount examination identified 1,008 of the 1,581 parasites identified by all methods. Had the direct wet mount microscopic examination been eliminated from this testing scheme, 45 parasites contained in 44 specimens would not have been reported. Of the protozoans identified exclusively by this method, all were in the cyst stage except one Entamoeba hartmanni isolate and one Endolimax nana isolate, which were identified by the trophozoite stage.

The concentration procedure was responsible for detecting 1,269 of the total number of parasites. This procedure detected 239 parasites contained in 224 specimens which were not detected by the other methods. Among the 104 protozoans identified solely by this method were 14 Entamoeba histolytica (14 C), 32 E. hartmanni (32 C), 28 Escherichia coli (2 T and 28 C), 21 E. nana (21 C), 7 Giardia lamblia (7 C), and 2 Chilomastix mesnili (2 C) isolates.

The trichrome staining technique detected 909 parasites which included 28 helminths, of which only 1 was exclusively identified by this method, and 880 protozoans. The protozoans exclusively identified were as follows: 10 E. histolytica (8 T and 2 C), 20 E. hartmanni (18 T and 3 C), 10 E. coli (8 T and 2 C), 33 E. nana (32 T and 7 C), 104 Dientamoeba fragilis, 18 G. lamblia (18 C), and 1 Iodamoeba buetschlii (1 T) isolates.

The recovery rates for the direct, concentration, and trichrome methods of 63.8, 80.3, and 57.5%, respectively, are not as noteworthy as their exclusive detection rates of 2.9, 15.1, and 12.5%, respectively.

The exclusive detection rate for the direct wet mount examination found in our study compares favorably with those recently reported. Estevez and Levine (2) reported that the direct wet mount was solely responsible for detecting only 3% of the parasites present, and Watson et al. (8) did not find any parasites exclusively by this method. We found that the direct wet mount added only 2.9% to the total number of parasites identified. Our results, however, should be viewed with caution for two reasons: (i) the study included only preserved specimens, and (ii) the distribution of helminths and protozoans may not be representative of that found by other institutions. Had the specimens contained predominantly the fragile trophozoite stage, the recovery rates for the direct and concentration procedures would have been lower because Formalin does not preserve this stage very well. Conversely, the detection rate for the trichrome staining of these specimens would have been higher, since the polyvinyl alcohol preservative would have prevented deterioration of the trophozoite.

If the direct wet mount examination had been eliminated from our testing protocol, it does not necessarily mean that the parasites which were exclusively detected by the direct wet mount examination would have had a negative impact upon the patient. We did not make any attempt to determine this or the number of specimens submitted by each patient. Therefore, eliminating this procedure from our testing regimen, provided that a concentration and a trichrome staining examination were performed, may not be clinically significant but may be cost-effective.

### LITERATURE CITED


