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Comparison of Direct Wet Mount and Trichrome Staining Techniques for Detecting *Entamoeba* Species Trophozoites in Stools

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Simulated freshly passed stools containing motile *Entamoeba moshkovskii* trophozoites were examined by direct wet mount and permanent (trichrome) staining techniques. The percentage of detection by direct mount was 4.8%. The percentage of detection of the trophozoites by direct mount plus permanent stain was 58.5%. Laboratorians should be cautioned not to rely solely on the direct wet mount for detection or identification of protozoan trophozoites.

It is well established that the fragile trophozoites of intestinal amoebae rapidly begin to deteriorate after passage (2, 8). Immediate preparation of a permanently stained smear upon arrival of the stool at the laboratory or immediate preservation of a portion of the stool in PVA-fixative or Schaudinn fixative is necessary to maintain trophozoites in the optimal condition. However, due to time and cost constraints, some laboratories still opt to rely on a direct wet mount of the fresh specimen for detection and identification of protozoan trophozoites, presumably via the motility of the organisms. In a comparative study, Scholten and Yang (8) demonstrated a much higher recovery of intestinal protozoans in preserved specimens than in unpreserved specimens. They concluded that the "prevalent practice of examining only 'fresh' stools" leads to many misdiagnoses. However, samples from this study generally were 1 to 2 days old when examined. A study by Garcia et al. (3) demonstrates the advantages of using a permanent stain over concentration procedures for detecting trophozoites in preserved stools. However, in this study, the specimens were preserved before receipt, and no direct wet mount was performed. Our study compares the direct wet mount and trichrome staining techniques for detecting amoebae trophozoites when the specimen is received less than 15 min after passage and can be examined immediately.

MATERIALS AND METHODS

A 2-day intestinal parasitology workshop was held five separate times at various locations in Utah. A total of 41 laboratorians, comprised of pathology residents, medical technologists, and other laboratory personnel who routinely perform parasitological examinations, attended. Several of the participants routinely performed only minimal parasitology testing and usually sent specimens to a referral laboratory for testing, although all participants had a general background knowledge of parasitology. During the course of the workshop, the participants examined direct wet mount preparations from a culture of actively motile *Entamoeba moshkovskii* trophozoites to acquaint themselves with the appearance, size, and motility of live amoebae trophozoites. *E. moshkovskii* is morphologically indistinguishable from *Entamoeba histolytica*, although *E. moshkovskii* occurs in sewage and has not yet been connected with any host (4). Participants also practiced preparing and reading permanent trichrome-stained smears containing amoebae trophozoites.

At the conclusion of the workshop, each participant was given a final unknown consisting of a carton containing simulated freshly passed stool that contained active *E. moshkovskii* trophozoites demonstrating typical progressive motility. Each participant also received a prepared unstained smear of PVA-fixed feces containing *E. moshkovskii* trophozoites. Each simulated freshly passed stool consisted of approximately 2 tablespoons (ca. 30 ml) of soft stool from a 10-month-old infant known to be free of intestinal parasites. The stool had been frozen at −70°C and thawed before distribution into cartons. Approximately 10 min before the unknowns were distributed, 0.5 ml of a 1-week-old culture of actively motile *E. moshkovskii* trophozoites in Balamuth medium (1), containing approximately seven organisms per oil immersion field, was mixed with each portion of stool. This mixture simulated a soft stool containing motile *E. histolytica* trophozoites, received for examination 15 min after passage. Before the workshops were conducted, a portion of simulated stool was examined by an instructor to assure that the active motility which is paramount for recognition of trophozoites among

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fecal debris was demonstrated by the cultured trophozoites in feces. Each carton containing a stool was given a different unknown number so that participants would not know that all specimens were identical. Because PVA-fixed smears require overnight drying, and Schaudinn-fixed smears require at least 1 h of fixing time at room temperature, the smears for permanent staining were prepared before the workshop to conserve valuable workshop time. The PVA-fixed material used to prepare the slides was the same as that used for the simulated fresh specimens (i.e., 2 tablespoons of soft infant stool with 0.5 ml of *E. moshkovskii* culture added) mixed one part to three parts of PVA fixative. The PVA-fixed smears contained approximately one organism per 20 oil immersion fields. Each smear was given a number corresponding to the number of the accompanying carton containing the fresh stool.

Participants were instructed to perform a macroscopic examination, a direct wet mount examination, and a Ritchie Formalin-ether (7) concentration procedure and examination on the fresh stool and perform a trichrome stain on the prepared smear.

The procedure of Melvin and Brooke (5) for direct wet mounts on fresh, unpreserved feces was used. A drop of physiological saline (0.85%) is placed on one end of a 3- by 2-in. (ca. 5- by 7.6-cm) slide, and a drop of iodine is placed on the other end. A small portion of feces is picked up with an applicator stick and emulsified in each solution. The density is monitored such that fine newspaper print can be read through the preparation. The preparations are placed under cover slips, sealed with Vaspar (Vaseline-paraffin mixed 1:1) and examined.

The Ritchie Formalin-ether concentration procedure was performed as described by Melvin and Brooke (5). The Wheatley trichrome (9) staining technique for PVA-fixed films was used for staining the prepared smears.

RESULTS

The results of the participants are compiled in Table 1. Only 2 out of 41 (4.8%) workshop participants detected the living trophozoites in the direct wet mount procedure. Neither of the two participants who detected motile trophozoites in the direct wet mount attempted to identify the organisms on the direct wet mount alone. Of 41 (58.5%) participants, 24 detected the trophozoites on the permanent trichrome stained smear. Of the 24 participants who were able to detect trophozoites on the trichrome stained smear, 18 identified the organism as *E. histolytica* trophozoites. *E. histolytica* was the expected correct answer, since *E. moshkovskii* is morphologically identical to *E. histolytica* and does not normally occur in the stool. Of the remaining six participants, four reported *Entamoeba* species trophozoites and two reported *Entamoeba coli* trophozoites.

TABLE 1. *Detection and identification results by wet mount and permanent stained smear*

Prepn	Detection (%)	Correct identification (%)
Wet mount	2/41 (4.8)	—[a]
Permanent stain[b]	24/41 (58.5)	18/24 (75)
Permanent stain after assistance[c]	15/41 (36.6)	11/17 (64.7)
Overall identification		29/41 (70.7)

[a] Neither of the two participants detecting trophozoites on wet mount attempted identification on wet mount alone.

[b] Two of these participants also detected trophozoites on the wet mount.

[c] If no trophozoites were found within 30 min, participants were shown one organism.

The remaining 17 out of 41 (41.5%) participants were unable to detect any parasites on either the direct wet mount or the permanent trichrome-stained smear. After a participant had spent at least 30 min scanning the stained smear under oil immersion (100×) with no parasites found, an instructor evaluated the staining quality of the slide and discretely found one organism for the participant. After being shown one trophozoite, 15 out of 17 participants were able to find several other trophozoites without assistance. Of the 17 participants who were shown one trophozoite, 11 were able to correctly identify the organisms as *E. histolytica* trophozoites. Of the remaining four participants, three reported *E. coli* trophozoites, and one reported *Entamoeba hartmanni* trophozoites. The percentage of detection of the trophozoites by permanent stain was 58.5%. (The two participants who detected trophozoites in both direct wet mount and permanent stain are included in the 58.5%). The overall percentage of correct identification by permanent stain was 70.7%.

DISCUSSION

A permanent trichrome-stained smear was found to be a much more effective method for detecting amoebae trophozoites in stools than the direct wet mount, even though the specimens were examined within 15 min after passage. In actual laboratory settings, stool specimens for parasitological examination may not always be examined within such a short time. Since trophozoites rapidly degenerate and lose typical motility after 1 to 3 h, detection may be rendered even more difficult in a real laboratory setting than was demonstrated in this study.

The fact that before attending the workshop many of the participants heavily relied on the direct wet mount for identifying trophozoites was demonstrated in their responses to a ques-

tion on a preworkshop examination. Nineteen of forty-one (46%) participants responded that motility on a direct wet mount was the only reliable method for identifying trophozoites from stool specimens. However, after studying the living culture of *E. moshkovskii* trophozoites, many of the participants expressed apprehension about the difficulty that they might encounter detecting motile trophozoites in the midst of fecal debris. Some participants, who had never before seen motile trophozoites, commented about the "slow" motility that the trophozoites exhibited. Possibly, the textbook term "progressive motility" was misinterpreted to mean "darting rapidly across the slide."

Four of the participants identified the trophozoites only as "*Entamoeba* species." This identification suggests that the specimen would be sent to a reference laboratory. If amoebae cannot be conclusively identified, a report of "*Entamoeba* species" or "unidentified amoebae" is much preferable to an attempted identification as to species.

This study was conducted retrospectively, based on observations of the results of the participants in the workshops. Certainly important parameters affecting the quality of parasitological examinations are the experience and proficiency of the parasitologists. Since all laboratorians with an interest in parasitology were encouraged to attend this workshop, the crucial factor of prior experience was not controlled. Therefore, the detection and identification percentages expressed in this study should not be used as an indication of parasitological skills in laboratories throughout the United States or throughout laboratories in Utah. Rather, the emphasis of this study should be placed on the large differences between the percentage of detection by direct wet mount and by the permanent trichrome stain.

Also, every participant's simulated specimen was not individually controlled to assure that the trophozoites put into the stool were demonstrating active typical motility, although a portion of simulated stool was examined before conducting the workshops to assure that cultured trophozoites would exhibit typical motility in stools that had been previously frozen. Theoretically, variations in the contents of an individual stool could have rendered trophozoites somehow undetectable in that stool, although there was no evidence to indicate that this was occurring. Spot checks by an instructor during the workshops revealed actively motile trophozoites in those direct wet mounts examined.

Additionally, freezing and thawing of the feces may artificially product changes in feces which influence trophozoite detection, although there was no evidence to indicate this. Further studies with freshly passed, genuine dysenteric stools and examiners of proven proficiency, although more difficult to conduct, would seem to be indicated.

We are not recommending that the practice of performing direct wet mounts be discontinued. This procedure is valuable for detecting cysts which may not concentrate well, such as *Giardia lamblia*, *Iodamoeba butschlii*, and *Hymenolepis mana* (6). Additionally, if motile trophozoites are seen, this rapid procedure may allow a prompt presumptive diagnosis. Instead, we caution laboratorians about relying solely on the direct wet mount for detection or identification of protozoan trophozoites. A permanent staining technique such as the trichrome stain is much more effective for detecting and identifying protozoan trophozoites in fecal specimens.

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