Evaluation of Intestinal Protozoan Morphology in Human Fecal Specimens Preserved in EcoFix: Comparison of Wheatley’s Trichrome Stain and EcoStain

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Received 6 November 1997/Returned for modification 24 February 1998/Accepted 15 April 1998

As a result of disposal problems related to the use of mercury compounds, many laboratories have switched from mercuric chloride-based Schaudinn’s and polyvinyl alcohol (PVA) stool preservatives to other, non-mercury-based preservatives. A comparison of organism recoveries and morphologies of the intestinal protozoa was undertaken with PVA containing the EcoFix zinc-based Schaudinn’s preservative (Meridian Diagnostics, Inc.); both Wheatley’s modification of Gomori’s trichrome stain (WT) and EcoStain (ES) were used to stain 51 human fecal specimens. Morphology, clarity of nuclear and cytoplasmic detail, overall color differences, and the ease or difficulty in detecting intestinal protozoa in fecal debris were assessed for the two permanent stained smears. Overall, organism morphology of the intestinal protozoa stained with WT and that of protozoa stained with ES were not equal in nuclear and cytoplasmic detail or range of color. However, the same organisms were identified in stained fecal smears with either WT or ES, with the exception of situations in which organism numbers were characterized as rare. Included were 67 protozoan challenges (number of organisms): Entamoeba histolytica-Entamoeba dispar (5), Entamoeba coli (9), Entamoeba hartmanni (6), Endolimax nana (12), Iodamoeba bütschlii (8), Blastocystis hominis (19), Giardia lamblia (6), Dientamoeba fragilis (2), yeast (2), and leukocytes (2). Five specimens were negative for parasites but contained fecal debris that was compared for morphologic detail and color range. The ES produces a more gray-green monotone with very little pink or red tone; contrast among the various colors is less than that seen with WT. Stain intensity for all organisms was acceptable, and there were no problems with stain deposition. The quality of the protozoan morphology with ES was often comparable to that with WT (36 of 67 [53.7%]) and, in some cases, better (24 of 67 [35.8%]). Organisms on the WT-stained smear exhibited better morphology in a few instances (4 of 67 [6%]), and in three instances, there were discrepant organism numbers.

For many years, Schaudinn and polyvinyl alcohol (PVA) fixatives with a mercuric chloride (HgCl2) base have been used to preserve stool specimens for the recovery and identification of intestinal parasites (1, 5, 8, 9). The concentration technique for either 5 or 10% formalin- or PVA-preserved specimens has been used for the recovery of helminth eggs and larvae and protozoan cysts. The permanently stained smear prepared from Schaudinn- or PVA-fixed material is used primarily for the identification of intestinal protozoa and is considered to be the most important technique for this purpose (2–4, 8, 10, 12). During the past few years, the issue of mercury disposal has become more important for clinical laboratories. Many facilities do not have the ability to dispose of small quantities of materials contaminated with mercury compounds. It is becoming almost impossible to find companies that will accept mercury-containing waste, and even if an appropriate company can be found, the cost is prohibitive. The use of Schaudinn’s fixative prepared with a compound other than mercuric chloride (HgCl2) would be advantageous. Several studies using the substitute copper sulfate (CuSO4) indicated that this compound did not provide consistent fixation for adequate protozoan morphology (5, 7). Some laboratories have switched to the use of sodium acetate-acetic acid-formalin (SAF) fixative coupled with the iron hematoxylin stain for the permanently stained fecal smears (11, 13, 15). Although this approach provides a good alternative, other laboratories want to maintain use of the trichrome stain. The combination of SAF fixative and trichrome stain may not always provide the same quality of results as those seen with the SAF-iron hematoxylin combination. Manufacturers have also begun investigating the possibility of providing non-mercury-based fixatives coupled with a trichrome-based stain that can be used as a combination approach, very similar to the combination of SAF and iron hematoxylin. The main objective of this study was to determine whether the same organisms preserved in EcoFix preservative, regardless of morphologic differences or numbers other than rare (see Materials and Methods), could be identified with either the EcoStain (ES) or Wheatley’s trichrome stain (WT) (14).

MATERIALS AND METHODS

Human fecal specimens (51 total) were collected in EcoFix preservative (Meridian Diagnostics, Inc., Cincinnati, Ohio). Of the 51 vials, 43 were positive for intestinal protozoa, 3 were positive for human cells and yeast, and five contained only fecal debris.

Two permanent stained smears were prepared from each vial, one smear being stained with ES according to the manufacturer’s directions (Meridian Diagnostics, Inc.) and the second smear being stained with WT (1, 3, 8, 10, 14). Both smears were examined by reviewing approximately 300 oil immersion fields (magnification, ×1,000), and the results were recorded by technologists other than those preparing the smears. This approach is generally more consistent than reading each smear for a set amount of time, since different individuals scan smears at different speeds (3, 10). Although the smears were identified as to stain used, without examination of the smears by microscopy, they could not be identified by gross appearance as being stained with ES or WT. Numbers of organisms and cells, clarity of nuclear and cytoplasmic detail, overall staining...
differences, and ability to detect organisms in fecal debris were assessed from the two permanent stained smears for both trophozoite and cyst stages and human cells. Although there were morphologic and color differences, stained smears were considered equivalent if the same organisms were identified in both specimens and the levels of nuclear and cytoplasmic detail were comparable. In cases where the number of organisms per smear was rare (no organisms per 10 oil immersion fields at a magnification of ×1,000 but at least one organism in the smear), we anticipated that there might be situations where one smear would be positive and the other smear would be negative. No discrepant results were anticipated with organism recovery and identification when organisms were characterized as more than rare.

RESULTS

A wide range of intestinal protozoa (67 protozoan challenges and cells were identified from the 46 positive specimens. In most cases, the comparative morphologies of the intestinal protozoa from ES- and WT-stained smears were equal (36 of 67 [53.7%]) (Table 1). In some cases, the ES-stained smears revealed better overall protozoan morphology (24 of 67 [35.8%]), and in a few cases, the WT-stained smears were better (4 of 67 [6%]). The overall color of ES-stained fecal smears is a gray-green or gray-blue monotone with very little pink tone, and the contrast among the range of colors is less than that seen with WT. Stain intensity among both ES- and WT-stained smears was acceptable, and there were no problems with stain deposition.

DISCUSSION

Although HgCl₂ has been used in the preparation of Schaudinn’s and PVA fixatives, other compounds have been used as substitutes in order to eliminate the many problems with disposal and cost inherent in the use of mercury compounds (5–7, 13, 15). The use of ZnSO₄ as a mercury substitute is gaining popularity. Many companies are manufacturing stool fixatives with ZnSO₄ in addition to other chemicals; these formulations tend to be proprietary (6). Recently, interest has also been expressed in developing stains that are formulated to be used with very specific fixatives for a combination approach to fixation and permanent staining.

Overall differences between WT and ES are related to the range of organism color, rather than stain intensity. Although there were differences in color and some differences in protozoan morphology, the majority of the smears stained with either ES or WT showed no significant differences that would influence the ability to recognize the organism. Studies have shown that when permanent WT-stained smears from fecal specimens preserved in HgCl₂ and ZnSO₄ were examined, the overall differences in recovery and morphology were minimal. However, protozoan morphology was superior in specimens preserved in HgCl₂, with clear, well-defined nuclear and cytoplasmic detail (6). Although the range of colors was present with WT (pink, red, purple, blue, and green), stained smears prepared from ZnSO₄-fixed material were more green and HgCl₂ smears were more uniformly blue with better differential colors (pink, red, and purple). However, organisms were detectable on both types of smears (6).

Definitive identification of intestinal protozoa frequently depends on the permanent stained smear, and it is important that this procedure be performed as a routine part of the ova and parasite examination (2–4, 8–10, 12). The ability to identify the organisms after staining depends on obtaining the best fixation as quickly after specimen passage as possible.

The first matched set of fixative and stain, EcoFix stool preservative and the ES permanent fecal stain, has been developed. Data from this study has confirmed that these two reagents used in combination provide an acceptable, and in some cases better, alternative to WT with fecal specimens preserved in EcoFix.

Some discrepancies are inevitable; sampling errors and organism shedding cycles contribute to the failure to always identify all intestinal protozoa in the stool sample, particularly when organism numbers are quite low. However, the laboratory community accepts these limitations as normal factors related to diagnostic test results in parasitology.

With more laboratories switching from mercury-based fixatives to alternatives, it continues to be important to match these fixatives with stains that provide the best possible morphology. Until present disposal and cost limitations on the use of mercury-based fixatives are modified, it is important to remember that any permanent stain will certainly increase the chances for protozoan recovery over that with concentration sediment alone (4). With the substitute fixatives and methods available at this time, the combination of EcoFix and ES provides a better alternative than that seen with EcoFix and WT.
formula. This study demonstrates the importance of developing matched stool preservative and stain combinations. As more fixative-stain matched sets become available, organism morphology may approach that of results seen with the “gold standard” combinations of mercury-based fixatives with either WT or iron hematoxylin stain. It is also interesting to note that proficiency testing fecal specimens used in the United States for diagnostic parasitology testing are all preserved in mercury-based fixatives. Currently, there are no plans to send proficiency testing fecal specimens preserved in any of the nonmercury fixatives to participants for testing in their laboratories. Until substitutes for mercury-based fixatives can provide the same day-to-day consistency in organism fixation and subsequent excellent morphology after staining, laboratories will continue to explore other reagent options.

REFERENCES