JOURNAL OF CLINICAL MICROBIOLOGY, July 2000, p. 2781–2783 Vol. 38, No. 7
0095-1137/00/$04.00+0

Performance of Three Enzyme Immunoassays and Two Direct Fluorescence Assays for Detection of *Giardia lamblia* in Stool Specimens Preserved in ECOFIX

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Received 17 February 2000/Returned for modification 19 April 2000/Accepted 1 May 2000

ECOFIX is a single-vial stool preservative that is both formalin- and mercury-free. We evaluated the abilities of three commercial *Giardia lamblia*-specific enzyme immunoassays (EIAs) (ProSpecT *Giardia* Microplate Assay [Alexon-Trend Inc.], Giardia Test [Techlab], and Premier *Giardia lamblia* [Meridian Diagnostics, Inc.]) and two commercial direct fluorescent-antibody (FA) assays for *G. lamblia* (Crypto/Giardia IF Test [Techlab] and Merifluor Cryptosporidium/Giardia [Meridian Diagnostics, Inc.]) to detect *G. lamblia* in 34 *G. lamblia*-positive and 44 *G. lamblia*-negative stool specimens (determined by traditional examination for ova and parasites) preserved in ECOFIX compared to their abilities to detect *G. lamblia* in the same specimens preserved in formalin as the "gold standard" for each assay. Of the 34 formalin-fixed positive specimens, the number detected by each assay was as follows:, Alexon EIA, 34; Meridian EIA, 27; Techlab EIA, 29; Meridian FA assay, 31; and Techlab FA assay, 28. Both FA tests and the Alexon EIA performed well with ECOFIX, but the other two EIAs detected fewer positive specimens (the difference was statistically significant with the Techlab EIA) when ECOFIX was the preservative. Use of *G. lamblia* cyst antigen from cultured organisms preserved in formalin and ECOFIX demonstrated that the Alexon EIA could detect smaller amounts of antigen in ECOFIX than the other two EIAs could and suggested that cyst antigen is more stable in formalin. We recommend that laboratories use an FA assay or the Alexon EIA if they plan to use ECOFIX as their stool preservative.

The increasingly stringent regulations governing the use and disposal of toxic laboratory reagents have sent many microbiologists in search of less hazardous preservatives for stool parasites. Formalin is a severe local irritant, and mercury compounds, found in polyvinyl alcohol (PVA), are local irritants and systemic poisons (5). Mercury disposal is tightly regulated and expensive, especially for high-volume laboratories. Zinc and copper-based PVA formulations have been evaluated as alternatives to the traditional mercury-based PVA fixatives (8, 9). Recently, single fixatives such as ECOFIX (Meridian Diagnostics), Parasafe (Scientific Device Laboratories), and Streck tissue fixative (Streck Laboratories) have been introduced to replace both formalin and PVA for concentration and permanent-stained smears of intestinal parasites (4, 7, 11, 12). Not only are these preservatives formalin- and mercury-free, but they also provide the convenience of a single-vial collection system for the preservation and transport of stool specimens for parasite examination. Although parasite morphology has been reported to be superior when the parasite is preserved in formalin and PVA, the single fixatives are generally thought to be adequate replacements for the traditional preservatives (4, 7, 11, 12). Streck tissue fixative has recently been compared to formalin and was shown to provide comparable results in an enzyme immunoassay (EIA) and a fluorescent-antibody (FA) assay for *Giardia lamblia* and *Cryptosporidium parvum*. We intended to evaluate how EIA and FA assay kits for the detection of *G. lamblia* in stool performed when the stool specimens had been preserved in ECOFIX. Because several published studies have evaluated the performances of these assays, we did not compare the performance of one assay to another in order to determine the performance characteristics of each one (1, 2, 6, 10, 13, 14). Our goal was to determine if ECOFIX interfered with the abilities of the EIA and FA kits to detect *G. lamblia* in stool specimens.

Stool specimens were obtained from patients at the Warren G. Magnuson Clinical Center, National Institutes of Health (NIH), Bethesda, Md. Specimens were received fresh, and portions were immediately placed into formalin and PVA. The remaining fresh stool was stored at 4°C. All specimens received an ovum and parasite (O&P) examination, which included sedimentation concentration with the Fecal Parasite Concentrator (Evergreen Scientific, Los Angeles, Calif.) and preparation of Wheatley's trichrome-stained smears, which were performed as described previously (5). When requested by the patient's physician, an EIA for the detection of *G. lamblia* antigen (ProSpecT *Giardia* Microplate Assay; Alexon-Trend, Inc., Ramsey, Minn.) was performed with a formalin-fixed stool specimen according to the manufacturer's instructions. The results from these studies were used to select unpreserved stool specimens from those previously stored for 5 days or less at 4°C to be placed into ECOFIX and formalin for testing with the EIA and FA assay kits. Thirty-four *G. lamblia*-positive specimens and 44 *G. lamblia*-negative specimens (as determined by O&P examination) from 78 different patients were tested. These preserved specimens were tested with three EIA kits (the ProSpecT *Giardia* Microplate Assay; Giardia Test [Techlab, Blacksburg, Va.], and Premier *Giardia lamblia* [Meridian Diagnostics, Inc., Cincinnati, Ohio]) and two FA assay kits (Crypto/Giardia IF Test [Techlab] and Merifluor Cryptosporidium/Giardia [Meridian Diagnostics, Inc.]) according to the manufacturer's instructions. When the specimens were

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TABLE 1. Detection of *G. lamblia* in positive stool specimens preserved in formalin or ECOFIX

Preservative	No. (%) of specimens positive				
	Alexon EIA	Meridian EIA	Techlab EIA	Meridian FA test	Techlab FA test
Formalin	34	27	29	31	28
ECOFIX	31 (91.2)[a]	18 (66.7)[a]	6 (20.7)[b]	30 (96.8)[a]	28 (100%)[a]

[a] *P* was not significant by Fisher's exact test with two-sided *P* value.
[b] *P* was <0.001 by Fisher's exact test with two-sided *P* value.

tested, tests with all three EIA kits were performed on the same day and tests with both FA assay kits were performed on the same day. The specimens were coded, and each assay was performed by a different individual who did not know the results of the other assays. The number of *Giardia* cysts on the FA assay slides was counted and was recorded when less than 10 organisms per slide were observed. Statistical analysis was performed by Fisher's exact test with a two-sided *P* value.

The three EIA kits were also tested for their ability to detect *Giardia* cyst antigen from cultured organisms placed in ECOFIX and formalin in the absence of stool. *G. lamblia* cysts were provided by Theodore E. Nash, National Institute of Allergy and Infectious Diseases, NIH, and were prepared as described previously (13). One milliliter of cyst suspension was placed into 2 ml of each fixative (neat), and twofold serial dilutions were made by using the fixatives as diluent. For each fixative dilutions of up to 1:8,192 were made. The three EIA kits were used to test the serially diluted cyst suspensions immediately after preparation and after 5 months of storage at room temperature.

We used the formalin-preserved specimens as the "gold standard" for each assay. All 44 negative stool specimens were negative with all five test kits. The Alexon EIA was least affected when specimens were preserved in ECOFIX because it detected 91.2% (31 of 34) of the positive specimens (Table 1). Although not statistically significant, the Meridian EIA detected only 66.7% (18 of 27) of the positive specimens when ECOFIX was used as the preservative. With ECOFIX as the preservative, the Techlab EIA detected only 20.7% (6 of 29) of the positive specimens, which was statistically significant. Both FA test kits performed well, as shown in Table 1. The one ECOFIX-preserved specimen that was false negative by the Meridian FA test could be explained by organism distribution since only eight *G. lamblia* cysts were observed in the formalin-preserved specimen.

Table 2 shows the performances of the three EIA kits with the *G. lamblia* cyst antigen from culture as the specimen. All of the EIA kits had comparable antigen titers as the endpoint (1:256 or 1:128) with both freshly preserved formalin-fixed antigen (0 months) and antigen stored for 5 months in formalin. The titer for the ECOFIX-preserved antigen at month zero was fivefold lower than that for the formalin-preserved antigen by the Meridian EIA and fourfold lower than for the formalin-preserved antigen by the Techlab EIA. The titer for ECOFIX-preserved antigen at month zero was twofold higher (1:1,024 versus 1:256) than that for the formalin-preserved antigen by the Alexon EIA; however, after 5 months the ECOFIX-preserved antigen titers decreased to 1:4 by the Alexon EIA and no antigen was detectable by the Meridian EIA or the Techlab EIA (Table 2).

Our results demonstrate that different EIA kits from various manufacturers perform differently when a stool preservative other than those recommended by the manufacturer is used. The Alexon EIA and Meridian EIA kits were designed to detect *G. lamblia* antigen in stool specimens preserved in formalin, sodium acetate-acetic acid-formalin (SAF), or merthiolate-formalin as well as unpreserved stool specimens. The Techlab EIA package insert indicates that specimens may be unpreserved or preserved in formalin or SAF. A recent evaluation of Streck tissue preservative demonstrated that it gave results comparable to those achieved when formalin was the preservative when specimens were tested by an Alexon EIA for both *G. lamblia* and *C. parvum* (11). We demonstrated that the Alexon EIA for *G. lamblia* also performs well when ECOFIX is used as the preservative. Perhaps Streck tissue preservative might not perform as well in EIA kits from other manufacturers, as we have demonstrated with ECOFIX. FA assays do not appear to be affected as easily by different preservatives (Table 1). Our findings for two FA assay kits from different manufacturers are similar to those previously published by Nace et al. (11), who found no difference in performance of the Meridian FA kit when formalin and Streck tissue preservatives were compared.

We cannot explain the differences in the performances of the three EIA kits when ECOFIX is used as the preservative. We compared the package inserts for the three assays in search of an explanation. The Alexon EIA and the Meridian EIA use polyclonal antibodies to capture the *G. lamblia* antigen and a monoclonal antibody in the detection reagent. The Techlab EIA uses a monoclonal antibody to capture antigen and polyclonal antibodies in the detection reagent. The difference in capture antibodies might contribute to the variation observed in our study. Polyclonal antibodies can interact with multiple epitopes, while monoclonal antibodies recognize only a single epitope. Therefore, the kits that use polyclonal antibodies as capture antibodies (Alexon and Meridian) may have an enhanced ability to collect *G. lamblia* antigen under all conditions. In addition, the Alexon detection reagent includes bovine serum albumin (BSA) which is not found in the detection reagents of the other two EIAs. Perhaps the addition of BSA allows the Alexon EIA to perform well in the presence of ECOFIX. Boone et al. (3) recently reported that both the Alexon and Techlab EIA kits detect cyst wall protein 1. This protein is reported to be highly stable, and our results seem to confirm cyst wall protein stability in formalin, yet the protein appears to be much less stable when it is stored in ECOFIX (Table 2).

We conclude that the performances of EIAs are more affected by stool preservatives than the performances of FA

TABLE 2. Titers of *G. lamblia* antigen in formalin and ECOFIX

Preservative	*G. lamblia* antigen titer					
	Alexon EIA		Meridian EIA		Techlab EIA	
	0 mo	5 mo	0 mo	5 mo	0 mo	5 mo
Formalin	1:256	1:128	1:128	1:128	1:256	1:256
ECOFIX	1:1,024	1:4	1:4	0	1:16	0

assays are. Laboratories that wish to switch to one of the new single-vial preservatives must evaluate the performance of the EIA or FA assay kit that they intend to use for the detection of fecal parasites in specimens preserved in the new preservative. *G. lamblia* antigen appears to be less stable when it is stored in ECOFIX, which could prohibit a laboratory from performing retrospective studies or surveys by an EIA with stored specimens if ECOFIX is used as the preservative. We suggest that the Alexon EIA or an FA assay may be the best choice for the detection of *G. lamblia* if ECOFIX is used as the stool preservative.

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