

Method

Detection of *Giardia lamblia* Cysts in Non-Fixed Long-Term Stored Human Feces by Direct Immunofluorescence Assay

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SUMMARY: *Giardia lamblia* cysts in fecal specimens are detected by conventional morphological methods. The direct immunofluorescence monoclonal antibody assay (DFA) is also applied to detect *G. lamblia* cysts in feces, but little is known about the usefulness of DFA in fecal specimens stored under various conditions. The aim of the present study was to evaluate the DFA for detection of *G. lamblia* cysts and to compare these results with the direct smear method in long-term storage of non-fixed fecal specimens. Fecal specimens with *G. lamblia* cysts were stored in a refrigerator at 5°C (14 samples), a freezer at -20°C (9 samples), or in 3.9% formalin-saline solution at room temperature (28 samples). *G. lamblia* cysts were detected by DFA in all stored specimens, while they were detected in only 56% of refrigerated and 93% of frozen specimens by the direct smear method. The storage period of all samples testing negative by direct smear was more than 24 months. Many degenerated cysts were recognized by DFA when cysts were negative by the direct smear method. Our results indicate that DFA is a useful method for detecting *G. lamblia* cysts in fecal samples that have undergone long-term storage.

INTRODUCTION

Giardia lamblia is the most common human intestinal parasite distributed worldwide, causing diarrhea, abdominal ailments, and weight loss in infected people (1). Identification of *G. lamblia* cysts is made based on morphological characteristics in fresh fecal specimens by conventional methods such as direct smear, concentration techniques using formalin-ethyl acetate, and cyst preparations stained with chlorazol black E or hematoxylin. Recently, fluorescent-labeled monoclonal antibody to the cyst-wall proteins became available for detection of *G. lamblia* cysts in feces (2). A recent large prospective study clearly showed an increased detection rate for *G. lamblia* cysts using the direct immunofluorescence monoclonal antibody assay (DFA) compared with conventional staining methods (3). Although subsequent reports generally support the improved sensitivity of DFA staining (4,5), clinical experience with DFA diagnosis of *Giardia* infections is limited thus far. Moreover, the detection of *G. lamblia* cysts in long term-stored feces by DFA has yet to be defined. The purpose of this study, therefore, was to evaluate the usefulness of DFA in detecting *G. lamblia* cysts and to compare results with DFA to those of conventional morphological tests of various stored human fecal specimens.

MATERIALS AND METHODS

Fecal samples: A total of 51 *G. lamblia* cyst-positive fecal specimens from 18 patients were collected at Kochi Medical School Hospital from April 1996 to January 2000. These fecal specimens were diagnosed positive for *G. lamblia* cysts by the direct smear method. Fecal samples were stored randomly in a refrigerator at 5°C (9 samples), in a freezer at -20°C (14 samples), or in 3.9% formalin-saline at room temperature (28

samples) for 30-48, 10-29, and 4-49 months, respectively.

Direct smear method: Stored fecal samples were mixed in a drop of saline on a clean slide and examined microscopically after placing then under a 18 × 18 mm cover slip. Specimens were examined for cysts of *G. lamblia* under a BX60 Upright Research Microscope (OLYMPUS, Inc., Tokyo) using 40× objectives.

DFA: DFA (Cyst-a-Glo™ kit; Waterborne, Inc., New Orleans, La., USA) was applied to detect *G. lamblia* cysts in each of the stored fecal specimens. Specimens were examined under a BX60 Upright Research Microscope using 40× objectives. A fluorescence microscope with a 470- to 490-nm exciter filter, a 505-nm dichromatic beam splitter, and a 515- to 550-nm barrier filter was also used. The frozen and refrigerated direct-smear fecal samples were classified into two groups: *G. lamblia* cyst-positive ($n = 18$ samples) and -negative ($n = 5$ samples). Degeneration rates with DFA results of these two groups were compared.

Morphological degeneration of cysts: A degenerated cyst was defined as a nonellipsoid-shaped cyst by DFA. The degeneration rate of *G. lamblia* cysts was determined as:

$$\text{Degeneration rate} = \frac{\text{Number of degenerated cysts}}{\text{Number of total cysts}} \times 100 (\%)$$

Number of degenerated cysts and total cysts were counted by DFA.

Statistical analysis: Statistical analysis was performed by the unpaired *t*-test for degeneration rate. A value of less than 0.05 was considered significant.

RESULTS

Using the smear specimens, *G. lamblia* cysts were detected in 100% of the formalin-fixed specimens by direct method, but only in 56 % of the refrigerated feces and 93 % of the frozen feces. DFA detected 100 % of the *G. lamblia* cysts in all three stored specimens. Cysts were detected by the direct smear method in fecal samples stored at various conditions

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Table 1. Detection of *G. lamblia* cysts by direct smear* and DFA**

Conservation period (Months)	Refrigerated (n = 9)		Frozen (n = 14)	Formaline (n = 28)	
	<24	24<	24<	<24	24<
Direct smear	3/3	2/6	13/14	14/14	14/14
DFA	3/3	6/6	14/14	14/14	14/14

*: Direct smear: Smear was examined under regular microscope.

** : DFA: Direct immunofluorescence monoclonal antibody assay.

for less than 24 months, but the storage period of all negative samples was greater than 24 months by the direct smear method. In particular, the detection rate of refrigerated feces was 33% (Table 1). Degenerated cysts were observed by DFA in samples testing negative by the direct smear method (Fig. 1). These cysts were not detected by bright field microscopy when the fluorescence optical path was closed (Fig. 2A, B).

DISCUSSION

Because Giardiasis breaks out as a result of waterborne or foodborne infection, storing and detection of *G. lamblia* cysts is necessary not only in fecal samples, but also in food or water samples. Moreover, when fresh feces cannot be obtained such as in cases where samples are transported from a distant area, feces are stored in the freezer or refrigerator for a relatively long period. In the present study, *G. lamblia* cysts were detected by DFA in all of the fecal samples stored under the various conditions, even when cysts were not identified by the direct smear method. DFA results were unaffected by the storage conditions for the samples or cyst degeneration, indicating that DFA has a high reactivity to *G. lamblia* cysts.

Erlandsen et al. has reported that cysts in fecal samples can be detected after cycles of freezing and thawing by immunofluorescence, even when the cyst wall cannot be seen

under bright microscopy (6). However, they did not examine the usefulness of immunofluorescence in samples stored for a long period. Our study has clarified the difference in the detection of *G. lamblia* cysts between the direct smear method and DFA in long term-stored feces kept in the refrigerator or freezer. Interestingly, formalin solution was found to be adequate for long-term storage of fecal samples. Formalin solution results in protein degeneration but does not have any influence on DFA reactivity.

Humans may excrete 150 to 20000 cysts/g into feces daily (7), but even under such conditions stool examinations may

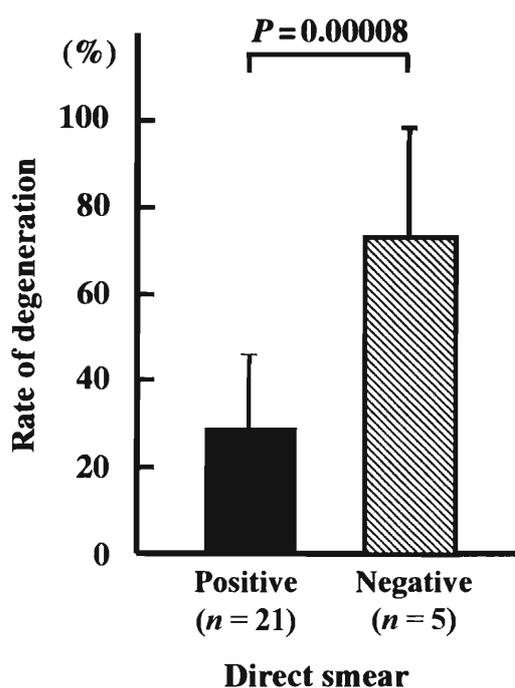


Fig. 1. Rate of degeneration of *G. lamblia* cysts. Results of the direct smear method were used to classify feces as *G. lamblia* cyst-positive or -negative, and these results were compared to those obtained by the identification of degenerated cysts by DFA.

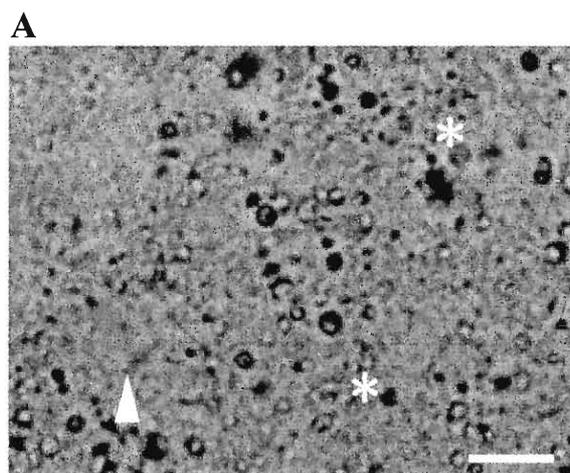


Fig. 2A. Bright field microscopy. Cysts were not detected by direct smear method (arrowhead). Scale bar = 10 μ m.

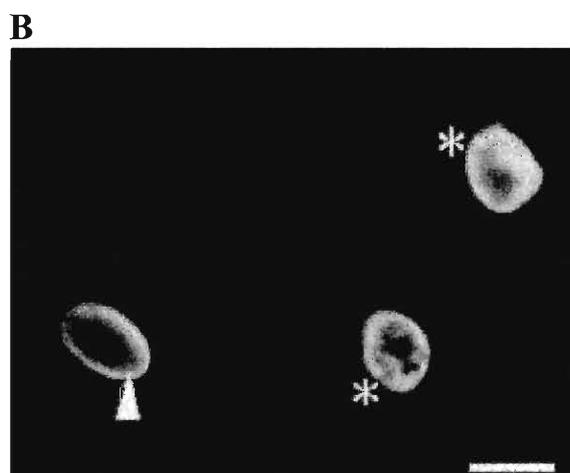


Fig. 2B. *Giardia lamblia* cysts detected by DFA in the refrigerated feces. Despite degeneration of many cysts after storage of more than 24 months, fluorescence was not different between typical-form (arrowhead) cysts and untypical-form cysts (*) in the same field as Fig. 2A. Scale bar = 10 μ m.

be negative by the direct smear method in some patients with chronic diarrhea, despite the presence of *G. lamblia* trophozoites in the duodenum (1). Moreover, we found either degenerated cysts or very few intact cysts even in fresh feces from individuals with *G. lamblia* infection. Degenerated cysts may not be detected by the direct smear method in these feces. Considering that the storage period and degeneration rate in fresh feces had no significant effect on cyst detection by DFA in various stored samples, it appears that *G. lamblia* cysts can be detected by DFA in cyst-negative samples by the conventional method when the immunological cyst wall is preserved.

In conclusion, DFA is a useful method for detecting *G. lamblia* cysts in long term-stored feces.

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