TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1992) 86, 164–165

Short Report

A capture ELISA detects *Giardia lamblia* antigens in formalin-treated faecal samples

A. J. Goldin[1], W. Apt[2], X. Aguilera[2], I. Zulantay[2], D. C. Warhurst[1] and M. A. Miles[1,*] [1]*Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK;* [2]*Southern Division of the University of Chile, Santiago, Chile*

The intestinal protozoan parasite *Giardia lamblia* has a worldwide distribution and a reported global prevalence of approximately 30% (PETERSEN, 1972). Giardiasis is endemic in developing countries and particularly affects children. In developed countries the parasite is commonly found in persons living in closed communities (YOELI *et al.*, 1972), in those who are immunocompromised (SMITH *et al.*, 1988), and in children attending day care centres (PICKERING *et al.*, 1984). There are also well documented outbreaks of giardiasis due to contaminated water supplies (GREEN *et al.*, 1990).

Diagnosis of giardiasis by microscopy requires examination of 3 successive concentrated faecal samples to achieve 95% sensitivity (WOLFE, 1979). Alternative invasive methods, such as duodenal aspiration, are neither practical nor 100% sensitive. We have recently reported the field evaluation in Chile of a highly sensitive (99%) and specific (96%) enzyme-linked immunosorbent assay (ELISA) for the diagnostic detection of faecal *Giardia* antigens (GOLDIN *et al.*, 1990). The collection and processing of faecal samples would be simplified if such an assay were effective with stools preserved in formol saline. We have now re-evaluated the capture ELISA with a bank of Chilean samples stored for 18 months, either at −20°C as suspensions in phosphate-buffered saline (PBS) or at 4°C as suspensions preserved in formalin.

The collection of stool samples from Chilean schoolchildren, microscopy of 3 formol-ether concentrates, and preparation of suspensions of 10% faeces in PBS (pH 7·2) or in 2% formalin/PBS were done as previously described (GOLDIN *et al.*, 1990). Samples from 161 children were tested in duplicate by ELISA with duplicate non-immune rabbit immunoglobulin (IgG) capture controls (GOLDIN *et al.*, 1990). All samples were tested on the same day and plates were read visually and by determining optical density (OD) at 492 nm. Final OD values were calculated by subtracting the mean OD of wells coated with non-immune rabbit IgG from the corresponding mean obtained with affinity purified anti-*Giardia* rabbit IgG. The cut-off value was set as the mean plus 3 standard deviations for those samples that were microscopically negative for parasites and commensals (colums C and C', Figure) after excluding 2 faecal samples that were strongly visually positive by the ELISA and also gave positive results with 2 alternative tests (ELISA from Trend Scientific, St Paul, MN 55112, USA and an indirect fluorescent antibody test, under development).

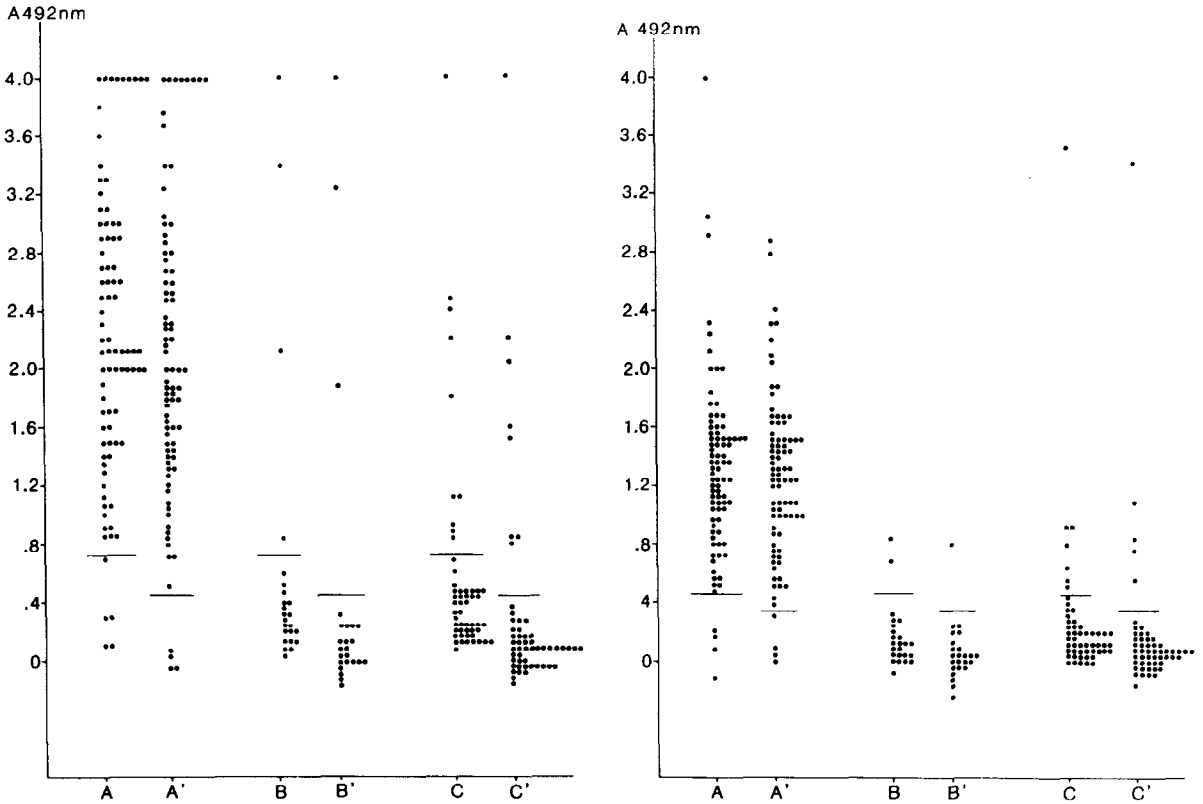

Figure. Immunodetection of *Giardia* antigens in stored faecal samples. Optical density at 492 nm (OD) is shown for enzyme-linked immunosorbent assays performed on stool specimens known to be microscopically positive or negative for *G. lamblia* and stored for 18 months at either −20°C in phosphate-buffered saline (left) or at 4°C in formol-saline (right). Each point represents the mean OD from a pool of 3 samples from one child. Vertical columns are labelled as follows. A: 85 samples containing *Giardia lamblia* cysts, 35 of which also contained cysts of either *Entamoeba coli* or *Endolimax nana*. B: 24 samples negative for *G. lamblia* cysts but containing other parasites (23 *E. nana*, 12 *E. coli*, 3 *E. histolytica*, 3 *Chilomastix mesnili*, 1 *Hymenolepis nana*) and 2 cases of faecal leucocytosis. C: 53 reference negative samples, with no parasites seen microscopically in 3 specimens from each child. Columns A', B', and C' show ODs for samples in groups A, B, and C after subtraction of control immunoglobulin G (IgG) values from wells coated with non-immune rabbit IgG. Hori-

*Author for correspondence.

Table. Assay performances in three series of tests[a]

	Microscopy		ELISA		ELISA–NSB	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
PBS 1988	95	97	99	96	99	96
PBS 1990	95	84	95	82	95	86
Formalin 1990	95	91	94	88	95	92

[a]Sensitivity=number of samples positive by microscopy and by enzyme-linked immunosorbent assay (ELISA) divided by number of microscopically positive samples. Specificity=total number of samples negative by microscopy and ELISA divided by number microscopically negative. –NSB=non-specific binding (see text) subtracted from ELISA value. PBS 1988=samples stored in phosphate-buffered saline at −20°C for 24–28 h, PBS 1990=samples stored similarly for 18 months, formalin 1990=samples stored in formol-saline at +4°C for 18 months.

The assay was clearly effective with formalin-treated faecal samples and, irrespective of storage methods, there was a clear separation between positive and negative specimens: formalin samples had lower ODs than those stored as suspensions in PBS (Figure). Sensitivity by visual interpretation was unchanged by storage but specificity fell somewhat, less so with the formalin samples than with those stored in PBS at −20°C (Table). Sensitivity with stored faeces was not improved by the measurement of ODs and specificity was slightly lower, but the specificity returned to equal to, or better than, uncorrected levels when subtractions were made for non-specific binding to rabbit IgG (Table). Overall, formalin-treated stools gave better assay performance than those stored in PBS at −20°C (Table).

During epidemiological surveys it is convenient to collect and transport faecal samples in formalin, and large numbers are often accumulated for testing as a single batch. This assay performed well on formalin-preserved material, although a small number of samples (4), which when fresh were considered positive by OD calculations, fell below the detection threshold of the assay. In contrast, a sample that was originally falsely negative was correctly diagnosed after prolonged storage in formalin. Two samples stored in PBS and one stored in formalin (columns B and B′, Figure) consistently gave positive ELISA results, although microscopy was negative. These samples probably reflect the greater sensitivity of antigen detection and are likely to be true positives. An apparent fall in specificity upon storage might therefore, in part, be due to the release of *Giardia* antigens from cysts below the threshold detectable by microscopy. We cannot entirely discount, however, the possibility that those false positives within the reference negative group (columns C and C′, Figure), other than the 2 excluded from the cut-off value calculation (see above), might be a result of storage conditions yielding hitherto undiscovered cross-reactive antigens in faecal samples that contain no *Giardia* antigen.

We conclude that formalin treatment and storage does not significantly affect the performance of this antigen detection ELISA, and this facilitates its application to large scale epidemiological surveys. Since non-specific binding increased in some microscopically negative samples, we recommend incorporation of non-immune capture controls for assays with stored sample collections.

We thank the Sir Halley Stewart Trust, the Godfrey Bradman Foundation, the Wolfson Foundation and the Racal Electronics Charitable Trust for financial support.

References

Goldin, A. J., Apt, W., Aguilera, X., Zulantay, I., Warhurst, D. C. & Miles, M. A. (1990). Efficient diagnosis of giardiasis among nursery and primary school children in Santiago, Chile by capture ELISA for the detection of faecal *Giardia* antigens. *American Journal of Tropical Medicine and Hygiene*, **42**, 538–545.

Green, E., Warhurst, D., Williams, J., Dickens, T. & Miles, M. (1990). Application of a capture enzyme immunoassay in an outbreak of waterborne giardiasis in the United Kingdom. *European Journal of Clinical Microbiology and Infectious Diseases*, **9**, 424–428.

Petersen, H. (1972). Giardiasis (lambliasis). *Scandinavian Journal of Gastroenterology*, **14**, 1–44.

Pickering, L. K., Woodward, W. E., DuPont, H. L. & Sullivan, P. (1984). Occurrence of *Giardia lamblia* in children in day care centres. *Journal of Pediatrics*, **104**, 522–525.

Smith, P. D., Lane, H. C., Gill, V. J., Manischewitz, J. F., Quinnan, G. V., Fauci, A. S. & Masur, H. (1988). Intestinal infections in patients with the acquired immunodeficiency syndrome (AIDS). Etiology and response to therapy. *Annals of Internal Medicine*, **198**, 328–333.

Wolfe, M. S. (1979). Giardiasis. *Pediatric Clinics of North America*, **26**, 295–303.

Yoeli, M., Most, H., Hammond, J. & Scheinesson, G. P. (1972). Parasitic infections in a closed community. Results of a 10-year survey in Willowbrook State School. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **66**, 764–776.

Received 21 March 1991; revised 22 July 1991; accepted for publication 23 August 1991