

# Comparison of techniques for detecting antigens of *Giardia lamblia* and *Cryptosporidium parvum* in faeces

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**Abstract**

**Aim**—To compare the use of commercial monoclonal antibody test systems—the Giardia CEL IF test and the Crypto CEL IF test—for the detection of *Giardia lamblia* and *Cryptosporidium parvum* antigens in faeces with conventional techniques.

**Methods**—Sensitivity and specificity were evaluated using preparations of cysts of *G lamblia* and purified oocysts of *C parvum*. Evaluation of 59 random faecal samples passing through the Department of Clinical Parasitology, Hospital for Tropical Diseases, London, was carried out for both organisms.

**Results**—The fluorescence staining techniques proved more sensitive than other tests routinely used for diagnosis.

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Giardiasis and cryptosporidiosis are two of the most commonly seen protozoal causes of diarrhoea. Outbreaks of diarrhoea have been frequently attributed to these organisms<sup>1-10</sup> and several methods of detection have been described.<sup>11-23</sup> But the diagnosis of *Giardia lamblia* and *Cryptosporidium parvum* infections remains problematic.

Traditionally, giardiasis has been diagnosed by microscopic detection of cysts or trophozoites in samples. Faecal samples are examined directly or with staining, with or without concentration. *G lamblia*, however, is often difficult to detect and faecal examinations frequently yield equivocal negative results. At times, symptoms of infection are present but parasites are not detected in the faeces.<sup>24</sup> In as many as 50% of infected patients parasites can not be demonstrated by a single faecal examination<sup>11</sup> and additional examinations are required for diagnosis. This is chiefly due to the extreme variability with which the parasite is excreted in both symptomatic and asymptomatic infections.

Current laboratory diagnosis of *Cryptosporidium* is generally by means of detection of oocysts in faeces, and occasionally in other specimens. Modified Ziehl-Neelsen staining is the method commonly used by the clinical laboratory.<sup>19,20</sup> Phenol-auramine staining is very reliable and is also widely used. Casemore and colleagues acknowledged that difficulties could be encountered distinguishing *Cryptosporidium* oocysts from non-cryptosporidial bodies, and concluded that no

single staining method was completely effective in detecting *Cryptosporidium*,<sup>17</sup> a view shared by others.<sup>25</sup> Microscopic techniques for detection of these protozoa in faeces can be laborious, insensitive, and prone to error if staff are not fully experienced. Fluorescence tagged monoclonal antibody staining has been reported to be more sensitive when compared with a non-immunofluorescence stain in the detection of *Giardia* antigen.<sup>26</sup> Machlauchlin *et al* reported that monoclonal antibody labelling of the *Cryptosporidium* oocysts provides a more rapid and accurate method of detecting the organism.<sup>21</sup>

Two commercial monoclonal antibody test systems (Giardia-CEL IF and Crypto-CEL IF; Bradsure Biologicals Ltd, Market Harborough, Leicestershire) utilise direct staining of acetone fixed specimens. The fluorescein labelled mouse monoclonal antibodies bind specifically to cell wall components of *G lamblia* cysts and *C parvum* oocysts.

The sensitivity of the Giardia-CEL IF test was evaluated by comparing it with direct microscopic examination of suspensions of faeces and also after the addition of Thomson's stain,<sup>27</sup> with or without concentration, by the formol-ether concentration technique.<sup>16</sup> The sensitivity of the Crypto-CEL IF test (CCIT) was evaluated by comparing it with conventional staining methods: Giemsa, modified Ziehl-Neelsen (MZN),<sup>17</sup> and phenol-auramine<sup>28</sup> using both purified oocysts and faecal samples.

**Methods**

Cysts of *G lamblia* and oocysts of *C parvum* were obtained from faecal samples submitted for routine parasitological examination at the Department of Clinical Parasitology, Hospital for Tropical Diseases, London. Purified oocysts of *C parvum* were provided by Dr V McDonald.

DETERMINATION OF THE SENSITIVITY OF DETECTION OF *Giardia Lamblia* CYSTS ANTIGENS

Using a faecal sample containing cysts of *G lamblia*, two standard methods for examination of faeces were used to compare the monoclonal antibody fluorescence method for detection of *Giardia* cysts (table 1).

A 1 in 10 dilution of unconcentrated faeces in saline was examined using direct examination of the saline suspension, and direct examination of the saline suspension with the addition of Thomson's stain. After formol-

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Table 1 Number of *G lamblia* cysts in 5 µl unconcentrated and concentrated faecal sample

Methods	Unconcentrated faeces	Concentrate from formol-ether concentration technique
Direct examination of wet preparation	1	35
Direct examination of wet preparation with Thomson's stain	12	35
Giardia-CEL IF test	36	318

Table 2 Number of *G lamblia* cysts in 5 µl of concentrates from 10 faecal samples

Specimen number	Direct examination of concentrate	Direct examination of concentrate with Thomson's stain	Giardia-CEL IF test
1	302	526	950
2	14	3	200
3	14	9	50
4	5	5	50
5	242	291	600
6	599	650	750
7	21	15	150
8	15	14	100
9	24	14	100
10	56	23	200

ether concentration of the same faecal sample, the deposit was examined using similar laboratory procedures.

Concentrated and unconcentrated faecal suspensions (5 µm) were used to prepare smears for the Giardia-CEL IF test. These were air dried and fixed in acetone for 5 minutes and stained according to the manufacturer's instructions.

Ten faecal samples (1-10) containing cysts of *G lamblia* were concentrated and 5 µl of the deposit was used to prepare smears which were examined in a similar manner (table 2). The total number of cysts present in each coverslip preparation was counted using ×400 magnification. The slides for the Giardia-CEL IF test were examined using ×400 magnification with a Zeiss Axioskop fluorescence microscope.

A further 20 random faecal samples, without prior knowledge of the parasitological findings, were examined in a similar manner, and the presence and absence of cysts noted (table 3).

Table 3 Random examination of unknown faecal samples for cysts of *G lamblia*

Number of faecal samples	No of samples positive for <i>G lamblia</i> cysts by:		
	Direct examination of concentrate	Direct examination of concentrate with Thomson's stain	Giardia-CEL IF test
20	3/20	3/20	5/20

Table 4 Number of *C parvum* oocysts in 5 µl of each faecal dilution by four staining methods

Dilutions (No of oocysts/ml)	Giemsas	Modified Ziehl-Neelsen	Phenol-auramine	Crypto-CEL IF test
1 in 100 ( $9 \times 10^2$ )	130	605	638	1151
1 in 200 ( $4.5 \times 10^2$ )	30	165	481	911
1 in 400 ( $2.3 \times 10^2$ )	18	105	207	502
1 in 800 ( $1.1 \times 10^2$ )	13	25	122	303
1 in 1600 ( $5 \times 10^1$ )	0	0	45	133
1 in 3200 ( $2.5 \times 10^1$ )	0	0	22	50
1 in 6400 ( $1.3 \times 10^1$ )	0	0	19	46
1 in 12 800 ( $6 \times 10^0$ )	0	0	11	11
1 in 25 600 ( $3 \times 10^0$ )	0	0	4	6

#### DETERMINATION OF THE SENSITIVITY OF DETECTION OF PURIFIED *C Parvum* OOCYSTS IN FAECAL SUSPENSION

Three standard methods for staining faeces were used in comparison with the monoclonal antibody fluorescence Crypto-CEL IF test (4) for detection of *C parvum* oocysts. The stains used were Giemsa (1), modified Ziehl-Neelsen (2), and phenol-auramine stain (3) (table 4).

A suspension (50 µl) containing about  $9 \times 10^7$ /ml of purified *C parvum* oocysts preserved in potassium dichromate was washed three times in phosphate buffered saline (PBS), pH 7.2. The pellet of washed oocysts was resuspended in 2.5 ml of PBS. This was mixed with 10 ml of 1 in 10 faecal suspension to make a dilution of 1 in 100. Further serial dilutions of 1 in 200 to 1 in 25 600 were made by doubling dilutions in saline.

Five microlitres of each faecal oocyst dilution were used to make smears which were air dried and fixed in acetone for 5 minutes before staining with methods 1-4. The total numbers of oocysts in 5 µl of each dilution were counted. Smears stained with methods 1-2 were examined at ×400 magnification using a light microscope. Smears stained with methods 3-4 were examined at ×400 magnification using a Zeiss Axioskop fluorescence microscope.

#### DETERMINATION OF THE SENSITIVITY OF DETECTION OF *C Parvum* OOCYSTS FROM FAECAL SAMPLES

Nine formalised (1 g/10 ml 10% formalin) faecal samples previously found to contain oocysts of *C parvum* were homogenised and 5 µl was used to prepare smears which were examined using the four stains described before. The total number of oocysts in each 5 µl of sample was counted (table 5).

A further 20 random faecal samples, without prior knowledge of the parasitological findings, were examined in a similar manner, and the presence and absence of oocysts noted (table 6).

Five formalised faecal samples previously found to contain oocysts of *C parvum* were homogenised and concentrated using two concentration methods: (1) standard formol-ether concentration technique; (2) modified formol-ether concentration method.<sup>17</sup> Two smears were prepared using 5 µl of well mixed deposit, air dried, and fixed in acetone for 5 minutes. Smears were also prepared directly from the homogenised faeces in a similar manner. All smears were stained using modified Ziehl-Neelsen, phenol-auramine, and CCIT and the total number of oocysts present was counted (table 7).

Table 1 shows the results from faecal samples examined for *Giardia* cysts with and without concentration. From the unconcentrated faecal samples, direct examination of saline suspension with the addition of Thomson's stain detected 12 times more cysts than the suspensions without Thomson's stain. The Giardia-CEL IF test, however, detected three times more cysts

Table 5 Number of *C parvum* oocysts in 5 µl of formalised faecal sample

Specimen number	Giemsa	Modified Ziehl-Neelsen	Phenol-auramine	Crypto-CEL IF test
1	53	63	103	129
2	8	15	127	78
3	8	4	6	7
4	8	10	34	17
5	63	198	450	467
6	45	80	130	211
7	10	28	27	52
8	35	213	355	566
9	33	60	96	633

Table 6 Examination of random faecal samples for oocysts of *C parvum*

Number of faecal sample	No of samples positive for <i>C parvum</i> oocysts by:			
	Giemsa	Modified Ziehl-Neelsen	Phenol-auramine	Crypto-CEL IF test
20	1/20	1/20	3/20	4/20

than the saline suspension with Thomson's stain. After concentration, the Giardia-CEL IF test detected almost 10 times more cysts than the other methods.

Table 2 shows that Giardia-CEL IF test has the greatest detection rate showing 10/10 samples with the highest number of cysts detected. The sensitivity of direct examination of concentrate and direct examination of concentrate with the addition of Thomson's stain in comparison with Giardia-CEL IF test was 41.0% and 49.2%, respectively.

Table 3 shows Giardia-CEL IF test detected five positives from 20 random faecal samples; by other methods three of 20 were positive. In comparison with Giardia-CEL IF test, the sensitivity of direct examination of concentrates and direct examination of concentrates with the addition of Thomson's stain were both 60%. Table 4 shows that the Giemsa stain was the least sensitive method for the detection of *C parvum* oocysts. Phenol-auramine and Crypto-CEL IF tests detected oocysts up to a dilution of 1 in 25 600; Giemsa and modified Ziehl-Neelsen could only detect oocysts up to a dilution of 1 in 800. The Crypto-CEL IF test detected twice as many oocysts as phenol-auramine. Except on the first dilution of 1 in 100, the

Crypto-CEL IF test detected five times more oocysts than modified Ziehl-Neelsen and 12 times more oocysts at a dilution of 1 in 800. The sensitivity of Crypto-CEL IF test over Giemsa staining was very much greater. In the 1 in 100 dilution Crypto-CEL IF test picked up eight times more oocysts than Giemsa. For the other dilutions, the difference was almost 30 times.

Table 5 shows that the two fluorescence methods detected the greatest number of oocysts in each faecal sample. The sensitivity of detection of the number of oocysts for each method in comparison with the Crypto-CEL IF test were 12.2% (Giemsa), 29.7% (modified Ziehl-Neelsen), and 61.5% (phenol-auramine).

Table 6 shows that the Crypto-CEL IF test detected four positives from 20 random faecal samples; by other methods one in 20 (Giemsa, modified Ziehl-Neelsen) and three/20 (phenol-auramine) were positive. The sensitivity of each method in comparison with Crypto-CEL IF test was 25-75%.

Table 7 shows the results of examining five direct faecal smears from known *C parvum* positive faecal samples compared using two concentration methods: (A) standard formol-ether concentration; (B) modified formol-ether concentration. After concentration with either methods A or B, the number of oocysts detected by fluorescence staining increased. With modified Ziehl-Neelsen staining, there were lower numbers of oocysts detected even after concentration (specimens 1, 3, and 4), indicating that the various stages of oocyst maturation affect the staining property. From these results, it was shown that concentration of faeces produces a significant increase in numbers of oocysts detected over direct faecal smears when stained by fluorescence methods. This is consistent with findings by other workers.<sup>10,29</sup> No cross-reactivity with the Giardia-CEL IF test was found with cysts of *Entamoeba histolytica*, *Entamoeba coli*, *Chilomastix mesnili* and *Endolimax nana*. No cross-reactivity with the Crypto-CEL IF test was found with oocysts of *Isospora belli*.

## Discussion

It is clear that the Giardia-CEL IF monoclonal fluorescence test for the diagnosis of giardiasis from faecal samples has far greater sensitivity of detection of cysts compared with other conventional methods with or without concentration. The preferred method for diagnosis of cryptosporidiosis is a fluorescence method with the modified Ziehl-Neelsen stain as a confirmation (confirming the recommendation of Casemore).<sup>17</sup> The use of modified Ziehl-Neelsen alone may result in missing oocysts in a light infection.

The performance of the phenol-auramine and CEL-IF test were sufficiently comparable to support the use of either method as a routine procedure, but the higher cost of Crypto-CEL IF test kit may limit its role to that of a confirmatory test.

Table 7 Comparison of the numbers of *C parvum* oocysts in 5 µl samples found by direct faecal examination and concentration techniques

Specimen number	Laboratory technique	Staining method		
		Modified Ziehl-Neelsen	Phenol-auramine	Crypto-CEL IF test
1	DFS	63	103	129
	FEC	33	134	195
	MFEC	85	106	147
2	DFS	0	6	7
	FEC	0	16	11
	MFEC	5	25	45
3	DFS	80	130	211
	FEC	50	170	440
	MFEC	28	232	421
4	DFS	28	27	52
	FEC	38	144	159
	MFEC	15	99	78
5	DFS	85	355	566
	FEC	119	890	1383
	MFEC	383	1332	1817

Keys: DFS: Direct faecal smear; FEC: Formol-ether concentration, MFEC: Modified formol-ether concentration.

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