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.

Giardiasis and cryptosporidiosis are two of the most commonly seen protozoal causes of diarrhoea. Outbreaks of diarrhoea have been frequently attributed to these organisms and several methods of detection have been described. But the diagnosis of *G. 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. In as many as 50% of infected patients parasites can not be demonstrated by a single faecal examination 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. 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*, a view shared by others. 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. Machlauchlin et al reported that monoclonal antibody labelling of the *Cryptosporidium* oocysts provides a more rapid and accurate method of detecting the organism.

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, with or without concentration, by the formol-ether concentration technique. The sensitivity of the Crypto-CEL IF test (CCIT) was evaluated by comparing it with conventional staining methods: Giemsa, modified Ziehl-Neelsen (MZN), and phenol-auramine 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-
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 × 107/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.17 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 uncentrated 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

<table>
<thead>
<tr>
<th>Specimen number</th>
<th>Giemsa</th>
<th>Modified Ziehl-Neelsen</th>
<th>Phenol-auramine</th>
<th>Crypto-CEL IF test</th>
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<td>9</td>
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<td>60</td>
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Table 6  Examination of random faecal samples for oocysts of C. parvum

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<th>Number of faecal sample</th>
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<td>3/20</td>
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Table 7  Comparison of the numbers of C. parvum oocysts in 5 μl samples found by direct faecal examination and concentration techniques

<table>
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<tr>
<th>Specimen number</th>
<th>Laboratory technique</th>
<th>Staining method</th>
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<th>Phenol-auramine</th>
<th>Crypto-CEL IF test</th>
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Keys: DFS: Direct faecal smear; FEC: Formal-ether concentration; MFEC: Modified formal-ether concentration.

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). 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.
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