Evaluation of Flow Cytometry as a Diagnostic Method for Detection of *Giardia lamblia* in Comparison to IFAT and Other Conventional Staining Techniques in Fecal Samples

Nancy M. Harba¹, Amany A. Rady¹, Khalid A. Khalefa²
Departments of Parasitology¹ and Clinical Pathology², Faculty of Medicine, Menoufia University

Received: April, 2012  Accepted: September, 2012

ABSTRACT

**Background:** *Giardia lamblia* is one of the most common diarrhea-related parasites in humans, where infection ranges from asymptomatic to acute or chronic disease. Because of the non-characteristic symptoms of giardiasis, as well as significant prevalence of cyst carriers, it is necessary to upgrade parasitological techniques for examination of feces, in order to avoid false negative results. Accurate diagnosis is important to exclude other parasitic causes of diarrhea.

**Objective:** The present study is designed to assess the efficacy of flow cytometry (FC) as a sensitive method for detection of *G. lamblia* cysts in stool samples in comparison with other standard conventional diagnostic methods.

**Subjects and Methods:** 70 patients (30 males and 40 females) ranging in age from 5 to 60 years were included in this study. Stool samples were taken from each patient on three successive days to detect *Giardia* cysts and evaluate the intensity of infection. Different methods were used that included concentration methods, permanent stained slides, immunofluorescent antibody test (IFAT) and FC.

**Results:** *Giardia lamblia* cysts were detected in 24 samples by IFAT with detection rate of 34.3%. Using trichrome stain 20 samples (28.6%) were positive, modified Ziehl-Neelsen Stain (MZN) detected 11 samples (15.7%), while Acridine Orange (AO) detected 14 positive samples (20%). Flow cytometry detected 18 samples with detection rate of 25.7%. Sensitivity and specificity of FC were 90% and 100%, respectively and IFAT sensitivity and specificity were 100% and 92%, respectively.

**Conclusion:** Results of the present study clearly demonstrated that incorporation of IFAT and FC can improve sensitivity of detection of *Giardia* cysts in stool samples. Although FC is more expensive than the other staining methods and IFAT, it is rapid, simple and accurate in estimating the quantity of parasites in each sample. Thus, FC can be recommended for detection of protozoa in stool.

**Keywords:** Flow Cytometry, IFAT, MZN, AO, Trichrome.

**Corresponding author:** Nancy M. Harba, nancyharba@yahoo.com

INTRODUCTION

*Giardia lamblia* is one of the most common parasites causing intestinal disease in humans and animals worldwide⁴. It is a bi-nucleated unicellular eukaryotic protozoan detected in the intestinal tract of humans and other mammals (birds, reptiles and amphibians). It affects the duodenum and the proximal jejunum³. This parasite has two phases in its life cycle, in which the flagellate trophozoite is the active form and is responsible for the clinical manifestations. The second form is infectious cysts that are excreted with stools and persist in the environment for several weeks⁶. Giardiasis typically occurs following the ingestion of water or foods contaminated with fecal material containing cysts; the infective dose may be as low as 10 cysts⁴. This parasite has been detected on vegetables and in water samples from field irrigation. Contaminated water appears to constitute a major route of contamination of fresh food, and acts as a potential vehicle of transmission⁴. Pathologic changes, as well as clinical manifestations of this condition, depend on numerous factors, the most important of which are the virulence of *G. lamblia* strain, number of inoculated cysts, age of the host and immune reactivity of the host at the moment of infection⁴. The most common presenting
Flow Cytometry in Detection of Giardia

Symptoms of giardiasis are diarrhea, abdominal pain, bloating, flatulence, malaise and severe weight loss resulting from malabsorption\(^7\). Patients may present with extra-intestinal symptoms, such as fever, maculopapular rashes, pulmonary infiltrates, lymphadenopathy, polyarthritides and urticaria\(^8\). The immune response to the parasite, promoted by introduction of *G. lamblia* antigens into the blood, and release of toxins initiating an inflammatory response, were offered as the most important causes for these extra-intestinal symptoms\(^9\). In developing countries, *G. lamblia* infection in children can interfere with growth and development\(^10\), and is considered as the most common cause related to growth retardation\(^11\). In Malaysia, *G. lamblia* was found to infect children less than 10 years old, with a prevalence rate of 15-20% particularly malnourished\(^12\). A lower overall prevalence rate of infection varying from 2%-5% was recorded in industrialized countries\(^13\). Recently, a survey was performed to demonstrate the prevalence of *Giardia* and *Cryptosporidium spp.* in rural and urban communities in North Delta, Egypt. The detection rate of *Giardia* spp. was 24.2\% being more frequent in children (under 12 year) than adults in both sexes\(^14\).

Conventional microscopy of three stool samples (with or without concentration techniques) is still being recommended as the reference standard (gold standard)\(^15\). Disappearance of the parasite cysts from fecal samples will not always mean cure of infected persons, because this parasite has periodic expulsion on alternative days or during various hours of the day. It also has a short latent time in some patients, and there is always a probability of its being hidden by bile pigments\(^16\). New methods have been investigated for automating the detection of *Giardia spp.*, including immunofluorescent assay and enzyme immunoassay\(^17\). The fluorescent dye used to stain the parasite easily differentiates it from fungi and bacteria when viewed by means of the fluorescent microscopy\(^18\). In the direct fluorescent antibody (DFA) fluorescent monoclonal antibodies bind to *G. lamblia* cysts with high sensitivity and specificity\(^19\). An enzyme-linked immunosorbent assay (ELISA) that detects excretory and secretory products of the organism is also available\(^20\). Methods of molecular biology like DNA probes and PCR, which are also recently employed diagnostic tests, are available in research centers but are not used for routine work\(^21\).

Flow cytometry is a measurement of characteristic single cells (cyto) suspended in a flowing saline stream, and is the only technique capable of rapidly identifying cells and parasites from large samples. It relies on scattering or fluorescence measurements that are made while the cells or particles pass through a capillary flow cell\(^22\). In parasitological studies it has been used for estimation of T- lymphocyte subpopulations in schistosomiasis and cytokine profile evaluation\(^23\). In another report FC was used in the diagnosis of *Cyclospora* in fecal samples and as a more precise method for the detection of *G. lamblia*\(^24\). It has been used in microbiology for detection of multiple organisms, and is used routinely in clinical pathology for differentiating leucocytic cells\(^25\).

The present study aimed to assess FC and IFAT as sensitive methods for detection of *G. lamblia* cysts in stool samples of diarrheic patients compared with other conventional diagnostic methods.

**SUBJECTS AND METHODS**

**Type of the study:** Descriptive analytic study.

The present work was performed during the period from March 2009 to September 2011 in Parasitology department, Faculty of Medicine, Menoufiya University, Egypt.

**Subjects:** Seventy patients (30 males and 40 females) ranging in age from 5 to 60 years, and attending outpatient and inpatient clinics of Tropical Medicine and Pediatric Department of Menoufiya University Hospital were included in the study for gastrointestinal problems. They variably complained of persistent diarrhea, flatulence, epigastric tenderness, crampy painful abdominal pains and copious light-colored greasy stools.

**Study design:** All patients provided medical history and underwent stool examination for detection of *G. lamblia*. Stool samples were taken from each one on three successive days and examined by different methods: direct wet mount and concentration methods; permanent stained slides; AO; IFAT; FC.

**Stool samples processing:** Each fresh fecal sample (~ 2 gm) was divided into 2 parts. One part (~ 1 gm) was processed immediately by mixing in phosphate buffer saline (PBS), centrifugation, followed by formal-ether concentration\(^26\). Concentrated specimens (~1 ml) were used to prepare smears for all staining procedures. The second part of the sample was processed by mixing in 5 ml of PBS, and filtering through cheesecloth. The filtered sample (1.5 ml) was mixed in 7.5 ml Sheather's sugar solution\(^27\) and centrifuged at 350 rpm for 5 minutes. The resulting interface and upper layer of liquid were transferred using a disposable pipette to a clean 10 ml tube for FC and immunofluorescence stain diagnostic procedures.

**Staining methods and calculation of intensity of infection:** Different stains employed were lugol’s iodine, MZN\(^28\), AO\(^29\) and trichrome\(^30\). The slides were observed under light microscope using low (X10) and high (X40) power magnifications for *G. lamblia*. For intensity of infection, 10 ul from the concentrated sediments were spread on a slide, stained by trichrome, and *Giardia* cysts were counted by high power field (X400). Three slides were prepared for each patient, counted, and the mean of readings was calculated. Intensity of infection is mild when number of cysts was <10; moderate: 10-20; severe: >20.
Immunofluorescent assay test (IFAT)\(^\text{30}\): From each sample a pellet (30 um) was spotted onto a microscopic slide and incubated at 37°C for approximately 3 min followed by the application of 40 um of a *Giardia*-specific monoclonal antibody solution (AbD serotec, Oxford, UK). The slide was incubated in a humid air chamber at room temperature for 40 min, briefly rinsed with PBS and allowed to air dry. Then 40 um fluorescein-isothiocyanate (FITC)-labeled solution (AbD serotec, Oxford, UK) was applied to the dried slide, kept in the dark for 20 min, rinsed with PBS and allowed to air dry. The slides were sealed with a glass cover slip. *G. lamblia* cysts were examined under a fluorescent microscope (Carl Zeiss, Jena, Germany) using a filter suitable for FITC detection (247 nm exciter filter, 510 nm selective beam splitter, 490 nm barrier filter and 249 nm additional filter). Positive control slides, containing formalin-fixed *G. lamblia* cysts were used for comparison and confirmation. The reaction was considered positive when the cysts showed apple green to yellow fluorescence.

Flow cytometry (FC)\(^\text{30}\): Two hundred ul of each sample was diluted 1:5 with PBS, and duplicate 200 ul aliquots were placed in 5 ml round bottom tubes. Twenty five ul of anti-*Giardia*-specific monoclonal antibody solution was added to each sample, and incubated in a humid air chamber at room temperature for 40 min. As an autofluorescence control 25 ul PBS was added to the duplicate tube. A control positive sample containing known numbers of *G. lamblia* cysts and control negative sample were examined prior to introduction of tested samples to calibrate the device including laser power, and to program it according to the fluorochromes used. Samples were examined by FACS Calibur\(^\text{TM}\) using monoclonal antibodies and fluochrome stain (FITC) specific for *G. lamblia*. Each tube was-washed by centrifugation in 2 ml PBS. The supernatant was discarded and 40 ul FITC was applied to each sample and kept in the dark for 20 min, mixed twice during incubation, and then washed with 2 ml of PBS and centrifuged at 1000 rpm for 5 min. The supernatant was discarded, and the pellets were analyzed using a flow cytometer (FACS Calibur\(^\text{TM}\) Becton, Dickinson). Every positive and negative control was analyzed in the same manner to ensure that any fluorescent debris did not appear in the analytic gate.

Interpretation of FC graph: The FACS detected parasites stained specifically with FITC as dots on a graph according to the cell volume, and the inner complexity of particles detected (Forward scatter, FSC; and side scatter, SCC). These dots are called events and the region surrounding these dots is called gated region on the screen of the computer.

Statistical analysis: For each technique (IFAT and FC), sensitivity, specificity, positive and negative predictive values and accuracy were calculated in comparison to trichrome stain as the standard stain for *G. lamblia* cysts. Chi square test was used for the presence or absence of association between qualitatively expressed relations. *P* value <0.05 was statistically significant. Pearson's correlation coefficient (r) was used to measure the strength of association between the two variables (quantitative variable). Positive correlation indicates that both variables increase or decrease together, whereas negative correlation indicates that as one test increases, so the other decreases. Therefore increase in number of cysts detected by trichrome stain is positively related to increasing number of cysts detected by FC (linear dependence). Significance determined by *P* value <0.001.

Ethical consideration: An informed consent was taken from all subjects. The study was approved by Research Ethics Committee, Faculty of Medicine, Menoufiya University, Egypt.

RESULTS

In the present study, 70 stool samples were collected and examined by different staining techniques to detect *G. lamblia* cysts. Trichrome stain detected 20 cases (28.6%), AO detected 14 cases (20%), while only 11 cases (15.7%) were detected by MZN stain (Table 1 and Figure 1). A total number of 24 samples were positive by IFAT with detection rate of 34.3%. *G. lamblia* cysts visualized by IFAT were oval, ranging from 8-12 um, in size and fluoresced bright green against the dark background. Flow cytometry detected 18 samples with detection rate of 25.7% (Table 2 and Figure 1). As regards the intensity of infection, IFAT was significantly effective in detecting mild intensity as it could detect 8 samples with detection rate of 33.3%, while trichrome stain detected only 2 cases (10%). Flow cytometry could not detect any cases with mild infection (0%), and detected all moderate and severe intensity cases that had been recovered by trichrome stain: 12 (60%) moderate and 6 (30%) severe. IFAT detected 11 (45.9%) moderate cases and 5 (20.8%) severe ones (Table 3). The validation of IFAT and FC in detection of *G. lamblia* cysts is summarized in table (4). There were 2 false negative samples in which *G. lamblia* cysts was detected by trichrome stain but not detected by FC with sensitivity and specificity of 90% and 100%, respectively. There were 4 positive cases detected by IFAT but not by trichrome stain with sensitivity and specificity of 100% and 92%, respectively. Regarding IFAT, all trichrome stain positive samples were confirmed by IFAT. It can be assumed that there were no false positive or false negative and the 4 samples not detected by trichrome stain may be considered as true positive with low intensity (no. of cysts < 5).

Pearson correlation coefficients were 0.89 (*P* < 0.001) and 0.98 (*P* < 0.001) for moderate and severe intensity of infection respectively indicating a positive association between FC and trichrome stain regarding intensity of infection.
Flow Cytometry in Detection of Giardia

Table (1): Comparison between different staining techniques for detection of *Giardia lamblia* cysts in stool samples (N=70).

<table>
<thead>
<tr>
<th>Staining methods</th>
<th>Positive <em>Giardia</em> cyst detection</th>
<th>Z test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichrome stain</td>
<td>20 (28.6)</td>
<td>0.37 *</td>
<td>0.72</td>
</tr>
<tr>
<td>MZN</td>
<td>11 (15.7)</td>
<td>0.25 **</td>
<td>0.43</td>
</tr>
<tr>
<td>Acridine orange</td>
<td>14 (20)</td>
<td>0.17 ***</td>
<td>0.40</td>
</tr>
</tbody>
</table>

*Comparison between trichrome and MZN, **Comparison between trichrome and acridine orange, ***Comparison between MZN and acridine orange. No significant relation was recorded between different staining techniques for detection of *Giardia lamblia* cysts in stool samples.

Table (2): Comparison between different diagnostic methods for detection of *Giardia lamblia* cysts in stool samples.

<table>
<thead>
<tr>
<th>Staining method</th>
<th>Trichrome stain</th>
<th>IFAT</th>
<th>FC</th>
<th>X2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>20 (28.6)</td>
<td>24 (34.3)</td>
<td>18 (25.7)</td>
<td>1.28</td>
<td>0.48</td>
</tr>
<tr>
<td>Negative</td>
<td>50 (71.4)</td>
<td>46 (65.7)</td>
<td>52 (74.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>70 (100)</td>
<td>70 (100)</td>
<td>70 (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No significant relation was recorded between different diagnostic methods (P>0.05).

Table (3): Comparison between different diagnostic methods regarding intensity of infection (mean cyst count by high power field).

<table>
<thead>
<tr>
<th>Intensity of infection</th>
<th>Trichrome stain</th>
<th>IFAT</th>
<th>FC</th>
<th>X2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild (&lt;10)</td>
<td>2 (10)</td>
<td>8 (33.3)</td>
<td>0 (0)</td>
<td>9.27</td>
<td>0.009</td>
</tr>
<tr>
<td>Moderate (10-20)</td>
<td>12 (60)</td>
<td>11 (45.9)</td>
<td>12 (66.7)</td>
<td>1.97</td>
<td>0.37</td>
</tr>
<tr>
<td>Severe (&gt; 20)</td>
<td>6 (30)</td>
<td>5 (20.8)</td>
<td>6 (33.3)</td>
<td>0.91</td>
<td>0.64</td>
</tr>
<tr>
<td>Total</td>
<td>20 (100)</td>
<td>24 (100)</td>
<td>24 (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Detection of mild intensity of infection by IFAT was statistically significantly higher in relation to other diagnostic methods. (P < 0.001)

Table (4): Validity of FC and IFAT in relation to trichrome stain as gold standard test.

<table>
<thead>
<tr>
<th>Trichrome stain (gold standard)</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>18</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>50</td>
<td>70</td>
</tr>
</tbody>
</table>

Sensitivity: 90%
Positive predictive value: 100%
Accuracy: 97%

<table>
<thead>
<tr>
<th>IFAT</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>20</td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>50</td>
<td>70</td>
</tr>
</tbody>
</table>

Sensitivity: 100%
Positive predictive value: 83%
Accuracy: 83%

Table (5): Pearson correlation between trichrome stain and FC results regarding intensity of infection.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Trichrome stain (Moderate =12)</th>
<th>Trichrome stain (Severe = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.89</td>
<td>0.98</td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

P< 0.001: Highly significant relation between the intensity of infection and detection rate of FC.
Figure (1): *Giardia* cysts from stool samples and stained with a) Iodine (X1000), b) MZN (X1000), c) Trichrome (×1000), d) IFAT (X1000), e) Acridine orange(X400).

Figure (2): Flow cytometry histogram a) gated region (R1) of a positive sample for *Giardia* cysts. b) Events representing *Giardia* cysts in lower right quadrant.

<table>
<thead>
<tr>
<th>Gate</th>
<th>Events</th>
<th>%Gated</th>
<th>%Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>6.57</td>
<td>6.57</td>
<td>6.57</td>
</tr>
</tbody>
</table>

Figure (3): Single histogram for analysis of gated areas (R3) to the right of the vertical line: a) Negative control with only 6.5% positive particles b) Positive control with about 81.7% positive particles for *Giardia* cysts.


**DISCUSSION**

*Giardia lamblia* is one of the most common intestinal parasites in humans. Each year, 500 000 new cases are reported and about 200 million people have developed symptomatic giardiasis. It is also considered as one of the main non-viral causes of diarrhea in developed countries (Asia, Africa and Latin America). Prevalence of *Giardia* infection in Egypt was found to be 34.6% by PCR, which correlates with the overall detection rate of 34.3% detected in the present study by IFAT. Another study from Cairo, Egypt, reported detection of 5.35%, 10.7% and 14.3% *G. lamblia* in stool samples by direct routine microscopic examination, formol-ether concentration and parasep (fecal parasite concentrator) respectively. From Saudi Arabia, a prevalence of *Giardia* infection ranging from 4% to 29% was reported. The high detection rate of *Giardia* cysts in our study may be attributed to selection of highly suspicious symptomatic patients.

The present study aimed to compare the conventional methods, IFAT with FC for detection of *G. lamblia* in human fecal samples. Trichrome stain proved to be the best stain for identifying *Giardia* cysts as it provided the highest detection rate in infected samples (28.6%). MZN staining method detected 15.7% and AO stain detected 20%. In another report, modified trichrome gave the best result for identifying *Giardia* cysts, and it was also able to detect spores of *Microsporidia*. Another report found the parasite cysts in 2.9% by routine microscopic examination. Other studies reported that the detection of cysts in feces by wet mount, and MZN was in 50% and 70% of patients respectively, after a single stool examination and in more than 90% after 3 stool examinations. Infection in stool samples obtained from children attending three public day care centers in the city of Botucatu, state of São Paulo, Brazil, revealed 63.3% detection rate by microscopic examination and immunofluorescence, and 68% by ELISA. For the serological examination the sensitivity was 82% and 72% and specificity was 70% and 39% respectively, where the immunofluorescence showed higher concordance with microscopic examination than ELISA. By native lugol examination and trichrome staining method a closely similar detection rate of 84% and 88.6% was reported.

A recent study was performed to detect *Giardia* cysts in 30 water samples, modified trichrome detected 16.6% positive samples while MZN and acridine orange each detected 10% of samples with 60% sensitivity. In the present study, the detection rate of *Giardia* cyst by staining techniques is considerably low and this may be attributed to variability in stain uptake and the age of cysts after prolonged storage (some samples were preserved in formalin while others were stained fresh). In the present study, IFAT proved to be the most sensitive method for detection of *G. lamblia* cysts compared to trichrome stain (sensitivity 100% and specificity 92%). There were 4 positive cases detected by IFAT but not by trichrome stain. It was observed that the fluorescent intensity of the stain in a few numbers of preparations was somewhat weaker for the organisms isolated by using the concentration techniques (formalin-ether and Sheather's sugar). This may be attributed to interfering substances in the concentrating reagents.

Different studies using monoclonal direct fluorescent antibody (DFA) test to detect *G. lamblia* cysts reported lower detection rate of 11% of samples with a sensitivity of 79.5% and specificity of 100% by DFA and reported the sensitivity of the test as 99.2%. Garcia and Shimizu used various commercial ELISA and DFA kits for diagnosis of giardiasis. They reported a sensitivity of 94-100% and specificity of 100% by ELISA, while both rates were 100% for DFA. A recent study performed in Egypt using IFAT to detect *Giardia* cysts among 362 infants < 3 years of age, revealed 29% specimens positive. IFAT allows rapid scanning of stained slides, minimal background auto-fluorescence or nonspecific staining and enhances the identification of *Giardia* cysts, but limitation of its use is the requirement for a microscope with epifluorescence capabilities, which may not be available in many hospital laboratories. In addition, this method is considerably more expensive than conventional staining methods.

In the present study, there was a highly significant relation (*P* < 0.001) between the microscopic trichrome stain results and those obtained by FL for the detection of cysts as well as regarding intensity of infection. When the concentration of cysts in the stool sample was increased as determined by trichrome stain (10-20 cysts, > 20 cysts), the intensity of fluorescence of FC was also increased, while no fluorescence was detected with cyst absence or low numbers of cysts. This observation is in agreement with another study that established the threshold of detection of *Giardia* cysts as 2 x 10^2 cysts/ml. Below this threshold limit, the fluorescence was not strong enough to allow the discrimination of cysts. The higher sensitivity (90%) and specificity (100%) of FC may be due to the considerably larger sample volume analyzed.
(up to 0.5 ml), and evaluation of cell fluorescence (cysts) by specific-labeled fluorescent monoclonal antibodies concentrations. In Egypt, evaluation of water pollution was done to detect contamination with protoza. The study was designed to evaluate FC for detection of *Cryptosporidium and Giardia* in 30 water samples in Alexandria, in comparison with the standard staining techniques (43). The researchers detected *Giardia* cysts in eleven of the samples (100% sensitivity and 76% specificity) and *Cryptosporidium* in all 30 samples (100% sensitivity and 100% specificity). Several other studies also recommended FC for routine identification of both these parasites in water. Medema et al. (47) used FC to identify both *Cryptosporidium* and *Giardia* parasites in water samples in comparison to FITC, and reported more positive samples (58.8% and 94.1% respectively) than fluorescence microscopy (40% and 82.3% respectively).

In the present study, it was noticed that there is a non significant difference in detection rate between IFAT and FC (34.3% and 25.7%, respectively). A probable cause for this difference may be due to the 1:5 dilutions of the samples prior to FC analysis, which may have decreased the potential sensitivity of the FC. Dilution of the samples was essential, to reduce concentration of debris thereby making it easier for the antibodies to bind to their target. Even more importantly, it was essential to clear samples of large particle’s debris that might clog the apparatus. Therefore, the use of gauze rather than paper filters prior to examination of samples was important and resulted in faster and efficient outcome.

In conclusion, the results of the present study clearly demonstrate that to increase the detection rate of *Giardia* cysts in stool over the conventional methods, incorporation of FC is advisable to improve detection sensitivity, as it is rapid, simple and accurate. Sample analysis takes only minutes, whereas microscopic examination is often a very time-consuming procedure; and a larger number of samples could be analyzed. More importantly because the method is largely automated the results are not influenced by an analyst’s levels of fatigue and expertise, as they may be with microscopy, and are not dependent on technician expertise for the evaluation of samples, as is the case in immunofluorescence microscopy.

**Author contribution:** AA Rady proposed the research idea, wrote the manuscript and shared NM Harba in sample collection and performance of wet mount and IFAT. Both authors shared KA Khalefa in processing samples for FC staining and analyzing FC data. NM Harba revised the manuscript.

**REFERENCES**


Flow Cytometry in Detection of *Giardia*

14. Samn KAM, Samn AAM, Abou El-Nour MF. A survey of *Giardia* and *Cryptosporidium* spp. in rural and urban community in North Delta, Egypt New York Science Journal; 2012, 5(3):49-54.


17. Degerli S, Ozcelik S. Diagnosis of giardiasis by indirect fluorescent antibody test (IFAT) and ELISA methods. Turkiye Parazitoloji Dergisi; 2002, 26:370-3.


Flow Cytometry in Detection of Giardia

Tissue flow cytometry as an alternative method in detection of Giardia in stool samples: A comparison with non-invasive test and various staining methods

Khalid Abd El-Moneim (1), Amani Ahmed Raddis (2), Nansy Mahmoud Hurb (3)

Faculty of Medicine, Menoufia University (1), Department of Clinical Pathology (2), Department of Parasitology (1), Faculty of Medicine, Menoufia University

Objective of the study: The aim of this study was to evaluate the use of flow cytometry as a method in detecting Giardia in stool samples compared with non-invasive test and various diagnostic staining methods.

Methods: This study aimed to assess Giardia in 30 stool samples collected from patients with diarrhea, aged between 70 and 80 years, for a period of 60 days. The diagnostic methods used in this study included various diagnostic staining methods, such as the direct test, the enterochromaffin test, and the Giemsa stain. The results showed that 24 samples were positive for Giardia using the direct test, 14 samples were positive for Giardia using the Hematoxylin and Eosin stain, and 15 samples were positive for Giardia using the Ziehl-Neelsen stain.

Results: The results showed that flow cytometry was more sensitive than the direct test and non-invasive test in detecting Giardia, with a sensitivity of 92% and specificity of 100%.

Conclusion: Flow cytometry and the non-invasive test are more sensitive than traditional methods in detecting Giardia in stool samples. Therefore, it is recommended to use these methods in the diagnosis of Giardia.