BRIEF COMMUNICATION

COMPARISON BETWEEN IMMUNOMAGNETIC SEPARATION, COUPLED WITH IMMUNOFLUORESCENCE, AND THE TECHNIQUES OF FAUST et al. AND OF LUTZ FOR THE DIAGNOSIS OF Giardia lamblia CYSTS IN HUMAN FECES

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SUMMARY

In the present study, the performance of Immunomagnetic Separation technique, coupled with Immunofluorescence (IMS-IFA), was compared with the FAUST et al. and Lutz parasitological techniques for the detection of Giardia lamblia cysts in human feces.

One hundred and twenty-seven samples were evaluated by the three techniques at the same time showing a rate of cyst detection of 27.5% by IMS-IFA and 15.7% by both Faust et al. and Lutz techniques. Data analysis showed a higher sensitivity of IMS-IFA for the detection of G. lamblia cysts in comparison with the techniques of FAUST et al. and Lutz.

The use of this methodology as a routine procedure enables the processing of many samples simultaneously, in order to increase recovery rate of G. lamblia cysts and reduce the time of sample storage.

KEYWORDS: Giardia lamblia; Giardiasis; Cryptosporidium; Immunomagnetic separation technique; Diagnosis.

Giardia is a flagellated intestinal protozoan parasite, responsible for causing disease in various animal species including humans. Considered the most common intestinal pathogen worldwide7, Giardia lamblia (syn.: Giardia intestinalis and Lamblia intestinalis) presents two forms: trophozoite and cyst during its biological cycle. Giardia infection occurs by the fecal-oral route through the ingestion of water or food contaminated with cysts4. The general estimated prevalence of giardiasis among children in Brazil is 28.5% in various geographic areas1; in some environments, such as nurseries schools, orphanages, hospital wards, etc, its prevalence can be still higher7. In both symptomatic and asymptomatic carriers cyst elimination is not continuous, but may last for long periods7.

Laboratory diagnosis for Giardia is based on stool processing by different techniques and light microscope examination. Examination of fresh diarrheal specimens is useful for trophozoite detection whereas the centrifuge-flotation methods (Faust et al., Sheather, etc.) or centrifuge-sedimentation (Ritchie) are more adequate for cyst detection. The smears can be stained by Lugol’s iodine or iron hematoxylin2. Antibody and antigen detection, by Enzyme-Linked Immunosorbent Assay (ELISA), is also used for diagnosis in humans, as well as the detection of the parasites in water samples10.

Immunomagnetic Separation coupled with Immunofluorescence (IMS-IFA), is a technique based on the use of magnetic beads conjugated to specific monoclonal antibodies against the cell surface markers of bacteria, protozoa, etc. Immunomagnetic separation accomplishes the isolation of these specific structures from their environment: water, feces, mud, etc, while IFA can confirm the identification of the isolated structures3,8,9,14. The IMS-IFA technique has been used in tests for Giardia spp. cysts and Cryptosporidium spp. oocysts in environmental samples in our laboratory5. This technique was previously standardized for Giardia lamblia and Cryptosporidium spp. diagnosis in human feces. This was performed by seeding the feces with an internal sample control, that consisted of C. parvum oocysts and G. lamblia cysts (99 ± 1 of each) labeled with Texas Red® (ColorSeed®, BTF Pty Ltd., Australia). This procedure allows artificially seeded oocysts and cysts to be distinguished, from those belonging to the sample. Since no significant differences between the recovery average of the two parasites in chi-square analysis11 were observed, the final option was to use an “external control” that consisted of a suspension of Cryptosporidium parvum oocysts (donated by Dr. Duncan Veal from Macquarie University, NSW, Australia – 10^6 oocysts/mL), prepared in the laboratory, as this was less expensive than the former.

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The aim of this study was to compare the IMS-IFA technique with Faust *et al.* and Lutz techniques for the detection of *G. lamblia* cysts in human feces. This study received approval from the Ethical Committee of Universidade Federal de Santa Catarina, SC (N° 005/2001).

One hundred and twenty-seven stool samples were obtained from children (one per child), between 2 and 12 years old, from a nursery school, Alfa Gente Vila Aparecida (77 samples), and from two hospitals, Hospital Infantil and Hospital Universitário da Universidade Federal de Santa Catarina (50 samples), in Florianópolis, State of Santa Catarina. Feces were fixed in formalin-sodium acetate-acetic acid (SAF) in a proportion of 1:5 feces SAF and stored at room temperature (20 °C) for no longer than 4 months. Stool samples were processed by Faust *et al.* and Lutz methods, by three students under the supervision of the responsible teacher, for cysts detection using standard protocols according to DE CARLI and by IMS-IFA technique as described by GREINERT (2001).

The magnetic beads (BIOMAGPolyscience Inc, Warrington, USA) used in this study were composed of approximately 1 μm (diameter) magnetic particles consisting of an iron oxide core with a silane coating. They are also commercially available with goat anti-mouse IgG covalently attached and were conjugated with specific monoclonal antibodies (Mab) at the laboratory. Briefly, magnetic beads were washed twice in 6 mL tubes in Bead Buffer (PBS added with 1% (w/v) BSA, 0.1% (w/v) SDS and 0.05% (w/v) Sodium Azide). The beads were incubated with hybridoma supernatants, for 16 hours in an orbital shaker (Mini-rotator, Glass-Col®) at 4 °C. These hybridoma supernatants contained Mab, raised against either the walls of *Giardia* spp. cysts (Mab G203) or the walls of *Cryptosporidium* spp. oocysts (CRY104). Mab G203 and CRY104 were generously donated by Dr. Duncan Veal from Environmental Pathogens Lab, Macquarie University, NSW, Australia (3.0 mL of cell supernatants and 3.0 mL of Bead Buffer). Afterwards, the tubes were placed in an adequate rack, containing a magnet. The supernatant was discarded and the beads were resuspended in 6.0 mL of Bead Buffer and stored at 4 °C.

For IMS-IFA assay (that was performed by one person, but the membrane examination under the microscope was performed by two different people) 1 mL of formalin-fixed human stool was suspended in two centrifuge tubes (tubes 1 and 2) containing 7 mL of Bead Buffer. In one of the tubes (tube 2), 10 μL of the standard *Cryptosporidium* oocyst suspension was added (positive-control). Tubes were vortexed for 30 sec and left on the bench for 40 min for spontaneous sedimentation. Thereafter, each supernatant was transferred to a 6 mL capacity tube, and then 200 μL of Magnetic Beads, coupled with G203 (tube 1) or CRY104 (tube 2), was added. The suspensions were incubated in an orbital shaker, for 30 minutes, and transferred to the magnet-containing rack, for bead separation during 15 minutes. These supernatants were transferred to two fresh tubes (1’ and 2’) and returned to the magnet-containing rack to ensure that all the magnetic beads were captured. After the two-step capture procedure and supernatant discarding, the beads were washed in 4 mL of Bead Buffer, resuspended in 4 mL of 0.1M glycine buffer, pH 2.2, and vortexed for two minutes. The suspension was agitated in orbital shaker for 15 min (acid dissociation), and finally placed on the magnetic shelf for Magnetic Bead capture for five minutes. Each supernatant containing either cysts or oocysts was filtrated through two 8 μm Nucleopore Membranes, (Whatman, New Jersey, USA) and the cysts or oocysts incubated for 5 min with 100 μL of anti-mouse IgG coupled with FITC (Sigma Inc, USA) 1:100 dilution in Bead Buffer (Fig. 1).

The membranes were covered with a slide glass and examined in a BX-40 FLA (Olympus®) fluorescence microscope (200 X and 400 X) for the presence of *Giardia* cysts and *Cryptosporidium* oocysts. For the seeded samples, the number of oocysts was counted in order to calculate the cyst recovery rate (Fig. 2). Data were analyzed by the Chi-square test ($\chi^2$).

IMS-IFA analysis of the 127 processed stool samples resulted in 35 (27.6%) positive samples of *G. lamblia* cysts and 92 negative samples. Using standard Faust *et al.* and Lutz methods, 20 samples (15.7%) were positive and 107 were negative. Twenty out of the 35 positive IMS-IFA samples, were confirmed by both Faust *et al.* and Lutz methods (Table 1).

IMS-IFA proved to be a very sensitive and specific technique for *G. lamblia* cyst detection when compared with the standard Faust *et al.* and Lutz methods ($\chi^2 = 5.22$, GL = 1, $p < 0.05$). In addition, the labeled cysts are very bright, turning their visualization easier thereby avoiding misinterpretations, since some artifacts can interfere in the correct
Comparison between Immunomagnetic Separation, coupled with Immunofluorescence, and the techniques of Faust et al. and of Lutz for the diagnosis of *Giardia lamblia* cysts in human feces.


Fig. 2 - Detection of oocysts of *Cryptosporidium* spp. (left) and cysts of *Giardia lamblia* (right) by Immunofluorescent Microscopy (FITC), 400X, in human feces.

Table 1

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Number</th>
<th>Positive Samples n (%)</th>
<th>Negative Samples n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMS-IFA</td>
<td>127</td>
<td>35 (27.6%)</td>
<td>92 (72.4%)</td>
</tr>
<tr>
<td>Faust et al.</td>
<td>127</td>
<td>20 (15.7%)</td>
<td>107 (84.3%)</td>
</tr>
<tr>
<td>Lutz</td>
<td>127</td>
<td>20 (15.7%)</td>
<td>107 (84.3%)</td>
</tr>
</tbody>
</table>

Although IMS-IFA was shown to be more sensitive than Faust *et al.* and Lutz methods, it is more costly. Consequently it is necessary to evaluate the advantage of its use and find ways of reducing costs. The use of this methodology as a routine procedure enables the processing of many samples simultaneously, in order to increase recoveries and reduce the time of sample storage.

**RESUMO**

Comparação entre a imuno-separação magnética, acoplada à imunofluorescência, e as técnicas de Faust *et al.*, e de Lutz para o diagnóstico de cistos de *Giardia lamblia* em fezes humanas.

No presente trabalho, o desempenho da técnica de Imunoseparação Magnética, acoplada à Imunofluorescência (IMS-IFA), foi comparado com aquelas das técnicas parasitológicas de FAUST *et al.* e de Lutz na detecção de cistos de *Giardia lamblia* em fezes humanas. Foram processadas 127 amostras de fezes pelas três técnicas paralelamente e a detecção de cistos foi de 27,5% para IMS-IFA e de 15,7% para as técnicas de FAUST *et al.* e de Lutz concomitantemente. A análise dos resultados mostrou maior sensibilidade da IMS-IFA na detecção de cistos de *G. lamblia* quando comparada aos métodos de FAUST *et al.* e Lutz.

A utilização desta metodologia como procedimento de rotina proporciona o processamento de várias amostras simultaneamente, além de aumentar a recuperação de cistos de *G. lamblia* e reduzir o tempo de estocagem das amostras.

**ACKNOWLEDGMENTS**

The authors gratefully acknowledge Dr. Maurício Sedrez dos Reis (Departamento de Fitotecnia/CCA, UFSC) for his assistance with the statistic analysis.

**REFERENCES**


Received: 8 April 2003
Accepted: 21 October 2003