INTRODUCTION

Cryptosporidiosis is a leading cause of persistent diarrhoea in developing countries due to the presence of Cryptosporidium Parvum in the gastrointestinal tract. This parasite is recognised as highly infectious enteric pathogen and is transmitted mainly by the faecal-oral route.

This pathology is particularly important to diagnose in immunocompromised persons, specially HIV-positive ones. Symptoms of cryptosporidiosis include watery diarrhoea, stomach cramps, weight loss, nausea and sometimes fever. In industrialised countries, 2 to 2.5% of persons admitted to hospital and suffering of diarrhoea eliminate oocysts. In AIDS patients, 10% of individuals are suffering from chronic cryptosporidiosis and this figure rises up to 40% in some undeveloped countries.

Diagnosis of Cryptosporidium Parvum is carried out by using either acid-fast (modified Ziehl-Neelsen method) or immunofluorescence staining on unconcentrated faecal smears. Several enzyme-linked immunosorbent assays are also available for detection of specific cryptosporidial oocysts antigens. New methods involving PCR may help to detect Cryptosporidium spp in water supplies or asymptomatic carriers. All these methods are sensitive but also time-consuming.

Coris BioConcept has developed an immunochromatographic test which enable to detect C. Parvum oocyst antigens in unconcentrated stool within 10 minutes. This test is compared with an ELISA test (TechLab, Inc, USA).

MATERIAL AND METHODS

103 human stools samples have been tested to compare the immunochromatographic test with ELISA. These samples come from patients suffering of enteritis. Sensitivity and specificity have been determined for immunochromatographic test versus ELISA. Stools samples were diluted approximately 5 times in Wash Buffer (PBS, detergent and 0.2% thimerosal) before their evaluation in both techniques.

ELISA (TechLab, Inc, USA)

96 wells microplates were sensitized with anti-Cryptosporidium Parvum oocysts antibodies. 100 µl of stools diluted 5 times in Wash Solution (PBS, detergent and 0.2% thimerosal) are incubated 60 minutes at room temperature on sensitized plates and then washed 4 times in Wash Solution. Detecting Antibody (Rabbit antibody to cell surface antigen of Cryptosporidium P.) are dispatched on the microplates before an incubation of 20 minutes followed by 4 washes with Wash Solution and then incubate 10 minutes with conjugate (anti-Rabbit IgG-peroxidase). The washing procedure is repeated (see above) before adding substrate and reading at 450 nm. A negative control is performed for each stool sample tested.

Immunochromatographic test

Nitrocellulose is sensitized with an immunoreactive directed against C. Parvum oocysts antigens. Specificity of immunologic reactions is performed by monoclonal antibody conjugated with colloidal gold particles. Sticks are immersed in stools diluted 3 times in dilution buffer provided with kit. Incubation between 5 and 10 minutes at room temperature can determine the presence of Cryptosporidium Parvum in samples tested.

RESULTS AND CONCLUSION

The comparison Crypto-Strip and ELISA specific for Cryptosporidium Parvum give the following results:

<table>
<thead>
<tr>
<th></th>
<th>ELISA</th>
<th>Crypto-Strip detection</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>POSITIVE</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>0</td>
<td>85</td>
</tr>
</tbody>
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Specificity = VN/(VN+FP)*100 = 100 %
Sensitivity = VP/(VP+FN)*100 = 94.4 %

Specifivity and sensitivity values observed between Elisa and Crypto-Strip validate the latter. Crypto-Strip test allows the reliable detection of oocysts in one step. By another way, diagnostic is obtained in less than 10 minutes, samples preparation included. Interpretation of results is non ambiguous and does not require any special skill and is simple to use. Following all those facts, we can conclude that Crypto-Strip is a specific, sensitive and easy to use test for diagnosis of Cryptosporidium Parvum oocysts in stools.

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