

Performance of an Immunoenzymatic Assay for *Cryptosporidium* Diagnosis of Fecal Samples

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We evaluated the diagnostic performance of a *Cryptosporidium* immunoenzymatic assay (ELISA). Fecal samples were collected from 94 HIV-seropositive patients. All specimens were processed with a commercially-available ELISA to detect *C. parvum* specific coproantigen and with a modified Ziehl-Neelsen stain (ZNm) microscope exam. Overall, sensitivity of the immunoenzymatic test was 100%, with a specificity of 96%; positive and negative predictive values were 89% and 100%, respectively. The commercial ELISA and ZNm proved to be valuable diagnostic tools for *Cryptosporidium* infection.

Key Words: Parasites, acquired immunodeficiency syndrome, *Cryptosporidium* diagnosis, Brazil.

Intestinal opportunistic parasitic infections are one of the most important causes of diarrhea [1], which is a serious health problem in tropical regions. Protozoan parasites, namely *Cryptosporidium parvum*, are important agents of parasite-induced diarrheal disease [2]. This parasite has been recognized as the pathogenic cause of diarrhea in AIDS patients [3], and it has been postulated that HIV-infected individuals may have their immune system activated by such parasites, thus affecting the progression of HIV disease [4]. Traditionally, laboratory diagnosis of *C. parvum* infection has relied upon the microscopic examination of fresh or preserved feces. However, microscopic diagnosis has several limitations, and recent studies have

concluded that stool antigen immunoassays equal or surpass the efficiency of microscopic detection of this microorganism [5]. We evaluated the diagnostic performance of a commercially-available immunoenzymatic assay for *C. parvum* diagnosis.

Ninety-four HIV-positive patients attending the Hospital de Base AIDS ambulatory in São José do Rio Preto, São Paulo, were chosen randomly and included in the study. Forty persons, employees of the Faculdade de Medicina de São José do Rio Preto (FAMERP), or their relatives, all apparently healthy, were included as controls. The samples were collected after a written informed consent was obtained from all individuals. This study was approved by the Research Board of the FAMERP.

Each participant was provided with a standard fecal collection vial containing 5% formalin and a spatula. All specimens were processed according to the immunoenzymatic assay (Alexon, Inc., BIOBRAS) instruction guide to detect *C. parvum* specific coproantigen [6]. The Modified Ziehl-Neelsen stain (ZNm) was also used to microscopically identify *C. parvum* in order to compare diagnostic procedures [7]. Statistical analyses were performed using EPIINFO version 6.0 statistical software. The chi-

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square test was applied to check for independence among the proportions. The significance level adopted for statistical inference was 5%.

The test performance of the *C. parvum*-specific coproantigen immunoenzymatic assay was assessed in terms of its sensitivity and specificity. None of the 40 control samples scored positive with either of the two techniques. Ninety-four paired samples were analyzed. The overall sensitivity of the immunoenzymatic assay was 100%, with a specificity of 96%; positive and negative predictive values were 89% and 100%, respectively (Table 1).

Cryptosporidium is not a new genus, but strong evidence suggests its emergence as an important community-disseminated protozoan pathogen, since this parasite has been frequently detected in untreated surface water, as well as in swimming and wade pools, day-care centers, and hospitals [8]. Furthermore, this coccidian is the leading cause of persistent diarrhea in HIV-infected individuals in developing countries, where infection rates range from 6 to 40% [9], which additionally contributes to community dissemination.

Table 1. Performance of a commercially available immunoenzymatic assay in diagnosing *Cryptosporidium parvum*, compared with a modified Ziehl-Neelsen exam

Immunoenzymatic assay	Modified Ziehl-Neelsen		
	Positive	Negative	Total
Positive	8	1	9
Negative	0	85	85
Total	8	86	94

Sensitivity: 100%; specificity: 96%, positive predictive value: 89%, negative predictive value: 100%.

The diagnosis of intestinal parasites in HIV-positive individuals was performed by microscopic examination of the stool, which has been recognized as the gold standard method for a long time [10]. Confirmation of the presence of *Cryptosporidium* in environmental and feces samples is labor-intensive, time consuming, costly, and often difficult, being dependent upon training and expert knowledge of morphologic differentiation of this small coccidian [11]. On the other hand, the ELISA kit is simple and rapid to use and offers a less subjective method than microscopy for detecting this protozoan in fecal samples submitted to a busy diagnostic laboratory [12]. This is a highly sensitive and specific technique, and it is useful for screening large numbers of specimens in a short time period. Also, it does not rely on microscopy skills [13].

Based on our data, both ELISA and ZNm techniques appear to be suitable for *Cryptosporidium* diagnosis.

Thus, we agree that the ZNm method continues to be a useful tool for screening tests of immunodeficient patients in clinical laboratories. Although the cost of the enzyme-linked immunosorbent assay is higher than that of ZNm, this new method allows cryptosporidial diagnosis, even when the parasite's integrity is compromised. Therefore, we believe that the ELISA test is a useful assay for ruling out cryptosporidiosis in immunocompromised individuals, especially when there are indicative clinical signs with inconclusive microscopic diagnosis, or in large-scale epidemiological surveys.

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