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False-Positive Results Obtained with the Alexon ProSpecT *Cryptosporidium* Enzyme Immunoassay

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***Cryptosporidium* is known to cause diarrhea in immunocompromised patients and is also associated with outbreaks of disease due to food-borne and waterborne parasites. Traditional procedures, involving iodine staining of wet mounts of stool sediments and trichrome staining, lack the sensitivity to detect *Cryptosporidium*. Special staining procedures, such as the modified acid-fast and safranin stains, are generally employed. Less labor-intensive antigen detection assays have simplified detection; however, careful attention to local epidemiology is important because false-positive tests occur. Here, we report two incidents involving 62 false-positive results obtained with the Alexon ProSpecT *Cryptosporidium* enzyme immunoassay, which were deemed false-positive based on negative results obtained from extensive microscopic examinations.**

Cryptosporidium is a coccidian parasite that continues to emerge as a significant enteric pathogen in immunocompromised patients as well as immunocompetent hosts (4). Infections are not uncommon in travelers and those working or living in agricultural environments and in children in day-care settings (1, 8, 10, 12). Additionally, large outbreaks of disease involving waterborne *Cryptosporidium* have occurred (7, 9).

Traditional parasitologic procedures, such as use of formalin ethyl-acetate concentrations with examination of iodine-stained preparations and trichrome staining, are not adequate to detect *Cryptosporidium* oocysts; therefore, special staining techniques, such as the modified safranin or acid-fast technique, must be employed. These procedures demand additional time and expertise yet fail to detect all infections (2). Commercially available, fluorescently labeled monoclonal antibodies (Meridian Diagnostics, Cincinnati, Ohio) significantly increase the sensitivity of direct microscopic examinations, but such examinations are still labor-intensive if large numbers of samples are being tested (6).

Use of enzyme immunoassays (EIA) greatly enhances laboratories' ability to rapidly screen large numbers of samples for the presence of *Cryptosporidium* and *Giardia* antigens in stool specimens. Overall, the sensitivities of these assays appear to be superior to traditional microscopy and are comparable to those obtained with immunofluorescent microscopy. However, problems with specificity, resulting in false-positive test results, are of concern and have been reported (3, 5, 11). Here we report two instances, each independent of the other, involving separate laboratories where significant numbers of false-positive *Cryptosporidium* results were obtained over a 4-month period with the Alexon ProSpecT EIA test kit (Alexon-Trend, Ramsey, Minn.).

Both laboratories employed the Alexon ProSpecT EIA test kit to screen stool samples for the presence of *Giardia*- and *Cryptosporidium*-specific antigens (GSA and CSA, respectively); however, one of the two laboratories routinely confirmed positive EIA results by fluorescent microscopy with the Meri-Fluor immunofluorescent assay (Meridian Diagnostics, Inc.). It was the latter protocol that revealed that false-positive results were likely being obtained.

Initially, nine samples were noted to be positive for CSA by EIA over a 6-week period. None of the positive results were confirmed by immunofluorescent staining (fluorescent-antibody [FA] staining) performed on concentrated samples. Repeat EIA testing was completed in all instances to rule out laboratory error in performing the assay. No technical errors were discovered, and all samples again tested positive. Staining by a modified safranin procedure also failed to reveal the presence of *Cryptosporidium* oocysts in all nine samples (13). Additionally, iodine-stained smears of the sediments were negative for parasites. In the following 2 months, 26 additional unconfirmed EIA-positive samples were identified, and while the positive EIA results were reproduced, confirmatory FA staining and modified safranin staining procedures failed to demonstrate *Cryptosporidium* oocysts.

The vendor was contacted after the initial nine samples could not be confirmed as true-positive samples. The problem was described and the results of confirmatory testing procedures were presented. Following these discussions, aliquots of the initial nine samples were forwarded to the vendor, along with EIA test kits and wash solutions currently in use. Five additional unconfirmed EIA-positive samples were later submitted.

The vendor completed EIA testing and blocking-antibody studies on the initial nine samples submitted. All were reported as EIA positive when tested in the vendor's quality control laboratory with retention kits with the same lot numbers as those used by the testing laboratory. Six of these samples were reported as confirmed positives based on blocking-antibody procedures; however, these studies utilized the same antibodies included in the test kit but lacked the chromogenic label. The remaining three samples could not be confirmed by these procedures and were therefore considered to represent false-positive tests. The five additional samples submitted to the vendor were reported as EIA negative by the vendor, so no blocking studies were pursued. However, these same samples were again tested by the referring laboratory, and all were

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found to be positive both visually and spectrophotometrically with optical density (OD) readings (minus the negative control OD) ranging from 0.524 to 1.407 (positive cutoff = >0.05 OD units).

The second instance of false-positive EIA testing occurred during the same time frame and involved stool samples from residents and employees of a long-term care facility with a history of diarrhea-like illness. Of 83 samples submitted, 36 (43.4%) tested positive for CSA. Confirmatory procedures, including FA staining, modified safranin staining, and conventional microscopy, were completed at a later date and failed to confirm the presence of *Cryptosporidium* oocysts in any of the 36 EIA-positive samples. Unfortunately, this information was available only after substantial time and financial resources had been expended investigating a suspected *Cryptosporidium* outbreak.

In a continued effort to resolve the discrepant results, now being noted in two different facilities, aliquots of the 14 samples previously sent to the vendor, along with 48 additional unconfirmed EIA-positive samples, were forwarded to a second biotechnology company that was developing its own *Cryptosporidium* enzyme immunoassay. All 62 samples were reported as negative for *Cryptosporidium* antigens by both visual and spectrophotometric readings.

In light of these results, the initial vendor pursued additional blocking-antibody studies but used antibody preparations with affinities for different *Cryptosporidium* epitopes. This data revealed that nonspecific reactions were indeed occurring in the current lot(s) of the *Cryptosporidium* ProSpecT EIA test kits and resulted in implementation of a product correction. New lots of the Alexon ProSpecT *Cryptosporidium* EIA were then made available, 5 months after we had first reported a suspected problem to the vendor. All 62 samples in question were retested with newly prepared lots of the ProSpecT *Cryptosporidium* assay. Negative results were obtained visually and spectrophotometrically for all samples, and the problem of false-positive test results has diminished.

Problems with the performance of diagnostic assays, and in particular with the washing steps of EIA procedures, should always be suspect when increased numbers of unexpected positive results are encountered. Technical error was rapidly excluded in this case because the initial EIA result was reproduced and positive results could not be confirmed by alternate reference procedures. Further, the problem was not being seen with a companion assay that was being performed at the same time but that detected GSA.

While unrelated to pertinent patient care issues, significant laboratory costs were incurred in resolving the false-positive EIA test results, including the costs of personnel time, repeat EIA testing, and additional confirmatory procedures. Unnecessary expenses surrounding infection control procedures resulted when the false-positive test results were given to the long-term care facility.

New testing methodologies continue to be developed, and some of them may rely on less technical expertise for the detection of traditional and emerging pathogens. Laboratory workers and clinicians must be cautious when interpreting results obtained from these types of assays and should not hesitate to question results which are unexpected based on clinical presentation and local epidemiology. The two incidents of false-positive *Cryptosporidium* antigen testing described here also demonstrate the value of routine confirmatory testing procedures, because such protocols can be beneficial in rapidly detecting problems with diagnostic assays. Local epidemiology, the expected clinical course of an infectious agent, and sensitivity and specificity data claimed in test kit package inserts are also useful in determining when expected thresholds are exceeded.

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