Evaluation of the Positive Predictive Value of Rapid Assays Used by Clinical Laboratories in Minnesota for the Diagnosis of Cryptosporidiosis

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We evaluated the positive predictive value (PPV) of rapid assays used by clinical laboratories in Minnesota to diagnose cryptosporidiosis. The overall PPV was 56% for rapid assays versus 97% for nonrapid assays; clinicians and laboratorians need to be aware of the low PPV of rapid assays when diagnosing cryptosporidiosis.

Cryptosporidiosis is a potentially serious diarrheal illness [1]. The number of reported cases in the United States has substantially increased recently [2], and a record 302 cases (5.8 cases per 100,000 population) were reported in Minnesota in 2007 [3]. One possible explanation for this observed increase is an increase in diagnostic testing due to the recent licensing of nitazoxanide for the treatment of cryptosporidiosis [2]. Immunochromatographic lateral-flow immunoassays (rapid assays) have previously been associated with false-positive Cryptosporidium results, leading to difficulties in public health surveillance [4, 5]. Therefore, more frequent use of rapid assays could artificially inflate reported cryptosporidiosis case numbers through the inclusion of cases with false-positive test results. To explore this potential effect, we evaluated the positive predictive value (PPV) of rapid assays used by clinical laboratories in Minnesota for the diagnosis of cryptosporidiosis.

Cryptosporidiosis is a nationally notifiable illness [2] and has been reportable in Minnesota since 1995. Laboratories in Minnesota are required to submit clinical materials from positive specimens to the Minnesota Department of Health Public Health Laboratory (MDH-PHL) in accordance with state communicable disease rules; confirmatory testing performed at the MDH-PHL is used to aid public health surveillance. Laboratories that submitted Cryptosporidium specimens to the MDH-PHL in 2008 were contacted to collect testing method information. All specimens received at the MDH-PHL were tested by polymerase chain reaction (PCR) [6]; if the results were negative, then the specimens were tested by MERIFLUOR DFA (Meridian Bioscience) and the modified Ziehl-Neelsen stain [7]. This confirmatory testing at the MDH-PHL was used as the basis for determining the PPV of testing performed at the submitting clinical laboratories.

In 2008, there were 235 cases of cryptosporidiosis reported to MDH; clinical materials were submitted from 179 cases (76%). Diagnostic tests used by the submitting labs comprised the ImmunoCard STAT! (Meridian Bioscience) (n = 126); the Remel Xpect (Thermo Fisher Scientific) (n = 16); 1 direct fluorescent-antibody (DFA) test, the MERIFLUOR DFA test (Meridian Bioscience) (n = 23); 1 enzyme immunoassay, the Wampole enzyme-linked immunosorbent assay (ELISA; Inverness Medical Innovations) (n = 1); and 1 microscopic visualization test, the modified Kinyoun acid-fast stained smear (n = 13). In this study, the Remel Xpect and ImmunoCard STAT! tests were considered to be rapid assays and the others nonrapid assays.

The MDH-PHL confirmed the presence of Cryptosporidium in 115 specimens, yielding an overall PPV of 64% for testing at the submitting laboratories. No samples that were negative by PCR tested positive by MERIFLUOR DFA or the modified Ziehl-Neelsen stain. Cryptosporidium was confirmed in 36 of 37 samples (PPV, 97%) that had been tested with a nonrapid assay at the submitting laboratory versus in 79 of 142 samples (PPV, 56%) that had been tested with a rapid assay (χ², 22.1; P < .001) (Table 1). The 64 specimens that tested negative at the MDH-PHL were submitted by 33 laboratories; of these, 25 (76%) used a rapid assay as their only method to detect Cryptosporidium.

During January–May, when the prevalence of cryptosporidiosis historically is low in Minnesota, Cryptosporidium was confirmed in 22 (39%) of 57 specimens received by the MDH-PHL. The nonrapid assays each had a PPV of 100%, whereas the PPVs of the 2 rapid assays were 33% and 34% (Table 1). During January–May, patients with an unconfirmed test result were less likely to report ≥1 major risk factor for cryptosporidiosis (contact with cattle, unpasteurized milk consumption, well
Rapid assays

For rapid assays, the PPVs did not differ significantly among specimens with known fixative type. Furthermore, among samples without preservation, accounted for only 16 (10%) of the 158 samples tested by rapid assays, the PPV did not differ significantly among specimens with known fixative type. In the low-prevalence season, the median period from specimen collection date to PCR test date was shorter during the low-prevalence season than in the high-prevalence season. However, the median period from specimen collection date to PCR test date was shorter during the low-prevalence season than in the high-prevalence season.

Table 1. Positive Predictive Value (PPV) of Results of Diagnostic Tests for Cryptosporidium Used by Clinical Laboratories in Minnesota, 2008

<table>
<thead>
<tr>
<th>Period</th>
<th>Modified Kinyoun acid-fast stained smear</th>
<th>Wampole ELISA</th>
<th>MERIFLUOR DFA test</th>
<th>Any Rapid assays</th>
<th>REMI Xpect</th>
<th>ImmunoCard STAT!</th>
<th>Any Rapid assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>January–May</td>
<td><em>(n = 1)</em></td>
<td><em>(n = 1)</em></td>
<td><em>(n = 2)</em></td>
<td><em>(n = 4)</em></td>
<td><em>(n = 6)</em></td>
<td><em>(n = 47)</em></td>
<td><em>(n = 53)</em></td>
</tr>
<tr>
<td>Low prevalence season</td>
<td><em>(n = 12)</em></td>
<td><em>(n = 1)</em></td>
<td><em>(n = 19)</em></td>
<td><em>(n = 31)</em></td>
<td><em>(n = 10)</em></td>
<td><em>(n = 67)</em></td>
<td><em>(n = 77)</em></td>
</tr>
<tr>
<td>June–October</td>
<td><em>(n = 13)</em></td>
<td><em>(n = 1)</em></td>
<td><em>(n = 23)</em></td>
<td><em>(n = 37)</em></td>
<td><em>(n = 16)</em></td>
<td><em>(n = 126)</em></td>
<td><em>(n = 142)</em></td>
</tr>
</tbody>
</table>

**NOTE.** Confirmatory testing at the Minnesota Department of Health Public Health Laboratory was used as the gold standard to calculate PPVs. The months of November and December are not included in either the low or the high prevalence season. DFA, direct fluorescent antibody; ELISA, enzyme–linked immunosorbent assay.

Patients with an unconfirmed test result (6 [20%] of 30) were more likely to be asymptomatic than those with a confirmed test result (2 [2%] of 82; \( \chi^2, 10.2; p = .001 \)). There was no significant difference between patients with a confirmed test result and those with an unconfirmed result as to whether they were treated with nitazoxanide (8 [50%] of 16 patients with a confirmed test result vs 10 [31%] of 32 patients with an unconfirmed test result) or were hospitalized (21 [19%] of 113 patients with a confirmed test result vs 9 [14%] of 63 patients with an unconfirmed test result).

Samples were received at MDH-PHL in a variety of fixatives, but the proportion confirmed did not differ by fixative type. Overall, preserved samples were more likely to be confirmed (98 [69%] of 142 preserved samples) than were unpreserved samples (7 [44%] of 16 unpreserved samples; \( \chi^2, 4.1; p = .04 \)); however, unpreserved specimens accounted for only 16 (10%) of the 158 specimens with known fixative type. Furthermore, among samples tested by rapid assays, the PPV did not differ significantly between unpreserved samples (6 [40%] of 15) and preserved samples (66 [61%] of 109; \( \chi^2, 2.3; p = .13 \)).

Overall, confirmed samples had a statistically significant shorter time from specimen collection date to PCR test date at the MDH-PHL than did unconfirmed samples (mean, 8.5 days vs 10.6 days; \( p = .02 \)). However, the median period from collection date to test date was shorter during the low-prevalence season than during the high-prevalence season for both confirmed and unconfirmed samples.

One potential limitation of this study was that the lack of statistical differences for some comparisons (eg, comparison of preservative type among rapid assay samples) may be simply a function of inadequate power. Also, the MDH-PHL confirmatory testing was not performed to provide diagnostic test results on individual patients, and time from collection to testing may have affected confirmation rates. However, the differences that were observed by test type, season, risk factors, and symptoms suggest that the differences between the PPVs of rapid assays and those of rapid assays were real. The lower PPV of rapid assays could reflect a variety of potential problems, including improper handling or storage of test kits in clinical laboratories, use of unapproved transport media, improper dilution of unpreserved specimens, operator error in performing the assay, misinterpretation of results, and/or problems with the antibody in the assay kits. Our results support the previous recommendations [9] that laboratories not use rapid assays as the sole method of diagnosing cryptosporidiosis. Clinical laboratories should ensure that they follow recommendations for sample collection, handling, and storage and proper test procedures when using these assays.

Previous studies about rapid assays have reported a sensitivity of 67.6%–98.8% and a specificity of 99.0%–100% [9, 10]. However, predictive values, both positive and negative, are a function not only of the sensitivity and specificity of the test but also, and more importantly, of the prevalence of the disease in the population tested. It is important that health care providers are aware of the limitations and proper use of rapid assays in the diagnosis of cryptosporidiosis and that they limit testing to patients who have symptoms characteristic of cryptosporidiosis. The low PPV of rapid assays, coupled with the large number of laboratories that use these tests as their sole method of...
diagnosing cryptosporidiosis, raises concern about the number of patients who are being given an inappropriate diagnosis of cryptosporidiosis and its corresponding treatment. Also, the widespread use of rapid assays could be artificially contributing to the increased number of reported cases of cryptosporidiosis.

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References