Evaluation of three commercial latex agglutination kits and a commercial enzyme immunoassay for the detection of cryptococcal antigen

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We compared the performance of the Meridian CALAS, Wampole Crypto-LA, Murex Cryptococcus latex agglutination assay, and the Meridian Premier EIA for the detection of cryptococcal antigen in serum and CSF. The assays demonstrated similar performance characteristics based on concordance values ≥93% but important differences were noted in endpoint titers.

Keywords Cryptococcus neoformans, latex agglutination, enzyme immunoassay

Introduction

Cryptococcus neoformans is an encapsulated fungus that is present in the environment (e.g., in soil, plant materials, and pigeon excreta) worldwide [1,2]. Cryptococcus neoformans is responsible for cryptococcosis, a primary pulmonary disease acquired through inhalation of dust contaminated with the organism. Its ability to grow at 37°C, the production of melanin, and the presence of a polysaccharide capsule contribute to the virulence of C. neoformans [3,4]. The incidence of cryptococcal disease is highest in immunocompromised individuals, especially in transplant patients [5,6] and in those with AIDS [7,8]. In immunocompetent hosts, infection can be either asymptomatic or can result in a mild pulmonary disease that can resolve spontaneously. However, in immunosuppressed individuals, the disease may disseminate from the lungs to the central nervous system, and cause a life-threatening meningoencephalitis [3,7]. Therefore, prompt diagnosis is necessary for the timely initiation of treatment. Detection of the C. neoformans polysaccharide capsule antigen is the primary serologic method in the diagnosis, since detection of anti-cryptococcal antibodies is often less sensitive and specific [2,9].

Latex agglutination (LA) tests for the detection of cryptococcal antigen have been available for over 40 years, but no direct comparison of commercially-available kits has been reported for a decade [10–14]. In this study, we compared the performance of three currently available commercial LA kits (CALAS Cryptococcal Antigen LA System (Meridian Bioscience Inc., Cincinnati, OH), the Murex Cryptococcus Test (Remel, Lenexa, KS) and the Crypto-LA test (Wampole Laboratories, Cranbury, NJ)) and a commercial enzyme immunoassay (EIA) (Meridian Premier Cryptococcal antigen) for the detection of C. neoformans antigen in serum and cerebrospinal fluid (CSF).

Materials and methods

A comparison of the three LA kits and the EIA was conducted using 100 (50 positive, 50 negative) archived sera (70) and CSF (30) specimens collected and stored at −70°C. Previous studies have demonstrated that storage at −70°C does not affect subsequent LA results [15]. Use of the stored sera and CSF for this study was approved by a Mayo Clinic Institutional Review Board. For our evaluation, the original results were based on testing performed by the Meridian CALAS LA kit. All LA testing was performed according the manufacturer’s instructions, including the use of pronase to remove potential nonspecific...
interference with the Meridian and Murex kits. Any endpoint titer, including 1:1, was considered positive for cryptococcal antigen. Testing by EIA was performed according to the manufacturer’s instructions using the automated Triturus EIA analyzer (Grifols-Quest, Miami, FL).

Results

Results are summarized in Tables 1 and 2. All 100 specimens were retested by Meridian LA and these data were compared to those obtained by Murex LA, Wampole LA and the Meridian EIA (Table 1). Due to the majority of specimens being submitted to our reference laboratory from outside hospitals, additional laboratory and clinical information was unavailable. Therefore, the data were analyzed to determine percent concordance (number of specimens showing agreement among all kits divided by the total number of specimens tested; Table 2). The results demonstrated a high concordance (≥93%) for all three LA kits, as well as excellent concordance (98.0%) between the Meridian EIA and the Meridian LA test (Table 2). Additionally, the kappa scores were greater than 0.81 suggesting near perfect agreement among the test results (Table 2). Interestingly, among the specimens showing discordant results, the endpoint titer was low (≤1:16) in the positive samples, similar to a previous study showing LA kit-dependent false-negative results at low cryptococcal antigen titers [16]. In contrast, specimens with an endpoint titer of ≥1:16 demonstrated 100% agreement among the LA tests (data not shown). Of note, the Meridian LA kit endpoint was felt to be the easiest to determine by the reading technologist.

The endpoint titers were also compared among the three LA kits. Agreement was defined as an endpoint titer within ±1 dilution of that obtained by the Meridian LA assay. The endpoint titers of the Wampole and Murex LA kits demonstrated agreements of 60% and 48%, respectively, when compared to titers obtained by the Meridian LA assay (Table 2). The endpoint titer of the Wampole and Murex assays showed 48% agreement when compared to each other (Table 2). The low kappa scores emphasized the slight or poor agreement among endpoint titers of the three different tests.

Discussion

The conclusions of this report are limited by the lack of available clinical information and supplemental laboratory results. However, our findings that the current versions of the three latex kits and the EIA provide concordant qualitative results are consistent with older reports comparing previous versions of the LA tests and EIA in various combinations. To our knowledge, our study is the first to compare the Murex LA to the Wampole LA and the Meridian EIA. Furthermore, our findings demonstrate that automating the Meridian EIA by using the Triturus analyzer yields results similar to those obtained by conventional LA testing.

In conclusion, the three cryptococcal antigen latex agglutination kits and the EIA demonstrated similar performance with concordances of ≥93% among the LA assays, and 98% between the Meridian LA assay and the Meridian EIA. Despite the excellent overall agreement in qualitative results (positive vs negative), the endpoint titers obtained by the three LA assays showed considerable variability. Therefore, it is important to note that cryptococcal antigen endpoint titers should only be directly compared (e.g., when following a patient’s response to therapy) when testing has been performed using the same LA assay. The Meridian EIA offers a more objective interpretation of results, and may be more suitable for large volume reference laboratories requiring automated testing.

Table 1 Comparison of the Meridian, Murex and Wampole LA tests and the Meridian EIA for the detection of cryptococcal capsular antigen.

<table>
<thead>
<tr>
<th></th>
<th>Meridian LA</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Murex LA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>42</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>51</td>
</tr>
<tr>
<td>Wampole LA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>44</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>52</td>
</tr>
<tr>
<td>Meridian EIA</td>
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<td></td>
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<tr>
<td>Positive</td>
<td>46</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>51</td>
</tr>
</tbody>
</table>

*N = 99 because 1 EIA result was ‘equivocal’ and was not repeated.
LA = latex agglutination. EIA = enzyme immunoassay.

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References


Table 2  Overall concordance and endpoint titer agreement among the three latex agglutination kits and the Meridian EIA for the detection of cryptococcal capsular antigen.

<table>
<thead>
<tr>
<th>Kit</th>
<th>Concordance (%)</th>
<th>Kappa1</th>
<th>Endpoint agreement (± 1 dilution)</th>
<th>Kappa1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wampole LA and Meridian LA</td>
<td>96%</td>
<td>0.920</td>
<td>60%</td>
<td>0.2</td>
</tr>
<tr>
<td>Murex LA and Meridian LA</td>
<td>93%</td>
<td>0.859</td>
<td>48%</td>
<td>-0.04</td>
</tr>
<tr>
<td>Wampole LA and Murex LA</td>
<td>93%</td>
<td>0.858</td>
<td>48%</td>
<td>-0.04</td>
</tr>
<tr>
<td>Meridian EIA and Meridian LA</td>
<td>98%</td>
<td>0.959</td>
<td>NA2</td>
<td>NA2</td>
</tr>
</tbody>
</table>

1. Results agreement by kappa scores are categorized as near perfect (0.81–1.0), substantial (0.61–0.8), moderate (0.41–0.6), fair (0.21–0.4), slight (0–0.2), or poor (< 0).
2. NA, not applicable.

LA = latex agglutination. EIA = enzyme immunoassay.

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