False-Positive Reactions in the Latex Agglutination Test for Cryptococcus neoformans Antigen

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The latex agglutination test for Cryptococcus neoformans antigen is a simple and rapid procedure for the diagnosis of cryptococcal meningitis. Although the test is sensitive, care must be taken to prevent contamination of the sample, which may result in false-positive reactions. It was discovered in our laboratory that immersion of a platinum wire inoculating loop into a sample of cerebrospinal fluid prior to testing introduced interfering substances leading to nonspecific agglutination. After further studies, it was determined that trace amounts of surface condensation (syneresis fluid) from agar, either added to the cerebrospinal fluid or adhering to the loop, were the probable source of contamination. It is suggested that the latex agglutination test for C. neoformans antigen be performed prior to culture or on a separate sample.

Cryptococcosis is a systemic infection caused by the fungus Cryptococcus neoformans. The organism typically gains entrance to the body by causing a lung infection and from there can rapidly disseminate, showing a preference for the central nervous system (11). Cryptococcal meningitis is one of the most devastating opportunistic infections suffered by patients with AIDS (9). Approximately 10% of patients with AIDS develop this infection, and nearly 60% of these patients die from the illness (3, 12).

The India ink technique is a time-honored approach for the direct detection of the encapsulated yeast cells of C. neoformans. Detection of the polysaccharide antigen of C. neoformans by means of antibody-coated latex particle agglutination (LA) has become an important adjunct in the diagnosis of cryptococcosis. As many as 90% of patients with cryptococcal meningitis may have antigen detectable in the cerebrospinal fluid (CSF) by LA tests (4). With the ready availability of LA kits for detection of this antigen, many laboratories have abandoned the India ink procedure because of its subjectivity and low sensitivity (8).

The Crypto-LA Test (Wampole Laboratories) has become a routine procedure in our laboratory as well as in many others. The test is reported to have a sensitivity of approximately 90% and to be nearly 100% specific when specimens are boiled and proper controls for nonspecific agglutination are included (2). The specificity of the cryptococcal latex test for antigen is obscured only by the presence of rheumatoid factor factors in the specimens, which may lead to false-positive reactions (5, 10). Although the immunoglobulin M anti-immunoglobulin G antibody, present in the serum of most patients with rheumatoid arthritis, can interfere with LA reactions, such antiglobulins are rarely found in the CSF and thus can account for few of the false-positive reactions obtained with CSF (7). Interpretation of results in the presence of interference factors is equivocal and confusing to the clinician (10). Boom and colleagues (2) suggest that when processing CSF, care should be taken to not contaminate the sample with the syneresis fluid (surface condensation) from chocolate agar plates, which, even in trace amounts, can produce false-positive LA test results. This reaction is heat stable and is not detected by the normal globulin controls.

It was noted in our laboratory that weak positive reactions had occurred in the undiluted CSF of several patients having no clinical evidence of meningitis, collagen vascular disease, or malignancy. False-positive results have in the past been attributed to the latter two conditions (1, 6). The specimens had been planted for acid-fast culture before removal of an aliquot of the sample for the LA procedure. Upon repeated testing with separate, untouched tubes of CSF, results were negative. We speculated that our false-positive reactions were due to material (perhaps syneresis fluid as described above) adhering to the inoculating loop being introduced into the specimen by simple immersion of the loop.

To test our hypothesis, we took three aliquots of normal CSF. In one aliquot, we immersed a wire inoculating loop; into the second tube, we immersed a sterile disposable plastic loop, and we put nothing into the third. All specimens were boiled for 5 min at 100°C before we proceeded. Results are shown in Fig. 1, with a positive control in row 1 and a negative control in row 2. Rows 3, 4, and 5 contain CSF exposed to the wire loop, CSF exposed to the plastic loop, and untouched CSF, respectively. Control latex sensitized with normal rabbit globulin, included to increase the specificity of the test, was added to each well in column 1. Reagent latex sensitized with anticytococcal globulin was added to each well in column 2. Control latex, which is sensitized with normal rabbit globulin and is included to increase the specificity of the test, was added to each well in column 2. The positive control results were as expected, being distinctly reactive in the reagent latex well and nonreactive in the control latex well. The negative control remained nonreactive in both wells. The well containing CSF exposed to the wire loop showed strong agglutination in the reagent latex well only. The CSF in remaining wells was nonreactive.

To further investigate our findings and to determine whether the reaction was due to the platinum itself or to material adhering to it, we tested the following materials in triplicate: syneresis fluid from the media used for acid-fast cultures in our laboratory, new loops and old loops (used for at least one year), and thoroughly cleaned loops. Results are shown in Table 1.

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It appears that agar syneresis fluid in trace amounts, including that burned onto the loop through long-term use, is responsible for the occurrence of false-positive reactions. After removal of the burned-on concrescence by thorough cleaning, no nonspecific reaction occurred. Boom et al. (2) speculated that the cross-reactivity may be due to contamination with agar of the cryptococcal antigen preparation used to immunize rabbits for commercial antisera.

To avoid the occurrence of false-positive reactions when testing for cryptococcal antigen, which might lead to inappropriate therapy with toxic antifungal agents, we have implemented the policy of performing the LA test only on CSF specimens before culturing or on separate tubes. It is also imperative to rigidly follow the manufacturer’s instructions to interpret as negative any sample that appears reactive in the Qualitative Protocol but is nonreactive in the 1:2 (or higher) dilution of the Quantitative Protocol (11).

TABLE 1. Nonspecific reactions in the cryptococcal antigen test by latex agglutination

<table>
<thead>
<tr>
<th>Material or medium</th>
<th>REAGENT LATEX</th>
<th>CONTROL LATEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syneresis fluid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diluted (10 μl of syneresis fluid + 100 μl of CSF)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lowenstein-Jensen media</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middlebrook 7H11 media</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Undiluted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowenstein-Jensen media</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Middlebrook 7H11 media</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Loops</td>
<td></td>
<td></td>
</tr>
<tr>
<td>New loop</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Old loop</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Cleaned old loop</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Plastic loop</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

We suggest that awareness on the part of the laboratory worker performing the LA test that this phenomenon occurs, as well as the implementation of measures to prevent it, will allow the continued use of this assay as a rapid and reliable aid in the diagnosis of cryptococcal meningitis.

REFERENCES
ERRATUM

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Cryptococcus neoformans Antigen

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Pathology and Laboratory Medicine, Brown University, Providence, Rhode Island 02912

Volume 29, no. 6, p. 1260, column 2, lines 27–30: “Control latex, which is sensitized with normal rabbit globulin and is
included to increase the specificity of the test, was added to each well in column 2” should be deleted.