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LETTER TO THE EDITOR

False-Negative Serum Cryptococcal Lateral Flow Assay Result Due to the Prozone Phenomenon

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Cryptococcus is a basidiomycetous yeast with worldwide distribution that has the ability to infect immunocompetent and immunocompromised hosts (1). The cryptococcal antigen (CrAg) test utilizing the IMMY, Inc., cryptococcal lateral flow assay (LFA) replaced the previously used latex agglutination assay in our laboratory in 2011 and is a method of simple and rapid diagnosis of *Cryptococcus* in blood or cerebrospinal fluid (CSF) specimens (2). As a serological assay, the LFA may rarely suffer from false-negative or very weak reactions in the setting of antigen excess. While this is more accurately termed a postzone phenomenon, we have chosen to use the term prozone phenomenon, as used in previous literature and the IMMY CrAg package insert (3). The test relies on the detection of glucuronoxylomannan, a component of the polysaccharide capsule present in both *Cryptococcus gattii* and *Cryptococcus neoformans* (2). We describe two instances of false-negative reports from sera that occurred recently at our laboratory, resulting in a change in our routine laboratory method.

Both cases were HIV negative and under investigation for large, unilateral pulmonary lesions. As part of the investigation process, both patients had serum cryptococcal antigen tests performed. These were conducted according to the manufacturer's instructions for qualitative determination, i.e., a 1:2 dilution utilizing 40 μl of specimen with 40 μl of diluent. Strips read at 10 min had no visible band in the test area. *Cryptococcus gattii* was isolated from both patients' sputa, and their sera were retested using the semiquantitative titration method to final endpoints of 1:655,360 and 1:327,680.

The IMMY CrAg lateral flow assay employs different dilution steps for qualitative (1:2) versus semiquantitative (1:5) titration. These cases demonstrate that the 1:2 dilution may occasionally produce a false-negative test result when antigen titers are very high, likely due to excess antigen binding colloidal gold in preference to immobilized antibody in the test area of the strip (4). The kit insert states that this is more likely above antigen concentrations of 0.14 mg/liter and recommends using the semiquantitative method when the prozone phenomenon is suspected (3), though in practice these instances are difficult to identify prior to testing. In each of our cases, positive results were obtained at the first semiquantitative dilution of 1:5.

Although this has previously been reported for CSF from HIV-infected patients (4), to our knowledge, this is the first reported instance of the prozone phenomenon occurring in the LFA when testing sera. It is notable that both these cases were HIV negative, had pulmonary disease only, and were infected with *C. gattii*.

In CSF, coincidental microscopy and culture routinely used in most laboratory settings are likely to detect undiagnosed cryptococcosis in the event of a false-negative CrAg test. Conversely, in serum specimens, a false-negative result may significantly delay the diagnosis if the CrAg assay is the only test employed. Because of this, our

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laboratory has now adopted an additional screening procedure using a greater dilution for serum tests, namely, 10 μl of serum, with 2 drops (80 μl) of sample diluent (i.e., at a dilution of 1:9). We recommend vigilance for similar phenomena in other laboratories utilizing this test.

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