Published in final edited form as:

AIDS. 2018 June 01; 32(9): 1201–1202. doi:10.1097/QAD.0000000000001805.

False-negative cryptococcal antigen test due to the post-zone phenomenon

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A 49-year-old man with a history of HIV-infection (CD4⁺ T-cell count: 45 cells/mm³), presented to Partners in Hope Medical Center in Lilongwe, Malawi with recurrent seizures, lethargy, reduced consciousness, and fevers. He was febrile, tachycardic, had elevated blood pressure, and was saturating 93% on room air. On exam, he was minimally responsive and had significant meningismus. A lumbar puncture revealed an increased opening pressure. Cerebrospinal fluid (CSF) analysis found 0 white blood cells, 0 red blood cells, 41 mg/dL of protein. CSF cryptococcal antigen lateral flow assay (CrAg LFA, IMMY, Norman, OK) was negative. India ink stain showed densely packed encapsulated organisms leading to the diagnosis of cryptococcal meningitis (Figure). For CSF, the IMMY CrAg LFA has 100% sensitivity (95% confidence interval [CI]: 96.0%–100%) and 100% specificity (95% CI: 97.0%–100%), except in cases with high CrAg concentrations (>0.140 mg/mL), i.e., the Post-zone Phenomenon. A semi-quantitative procedure of the CSF yielded the following titers: 1:5, negative; 1:10, weakly positive; 1:20, positive; 1:40, weakly positive; and 1:80, negative. Amphotericin B and high-dose fluconazole were initiated.

CrAg LFA

The IMMY CrAg LFA is an antigen-capture assay that detects free capsular CrAg from serotypes A, B, C, and D (*Cryptococcus neoformans*, serotypes A and D; *Cryptococcus gattii*, serotypes B and C) [1, 2]. The highly sensitive and specific assay uses two monoclonal antibodies, one for serotypes A, B, and C, and the other for serotypes A and D [1]. The test is a lateral flow immunochromatographic assay platform—a dipstick assay [3].

Post-zone Phenomenon

The Post-zone Phenomenon is a false-negative test caused by excessive antigen, relative to antibodies, which prevents antibody-to-antigen crosslinking [4]. Crosslinking is necessary for immunochromatographic detection [4]. That effect is often mistakenly attributed to the

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Competing Interests

The authors have no competing interests to declare.

Prozone Phenomenon (Hook Effect), which is a false-negative test caused by excessive antibodies relative to antigen [4]. When compared to a reference standard, the IMMY CrAg LFA had a sensitivity of 100% when samples suspected of having a high organism load were diluted, however the assay had a sensitivity of 90.9% when samples were not diluted [3].

A prior case was reported of unencapsulated *C. neoformans* that was CrAg negative [5], however we observed competent capsules on microscopy. Our patient's CSF tested CrAg positive after dilution, confirming the Post-zone Phenomenon. Among cases with a high suspicion of cryptococcal meningitis and a negative CSF CrAg LFA, serial dilutions must be performed to avoid false-negative test results and significant morbidity and mortality to the patient.

Acknowledgments

The authors would like to acknowledge the staff at Partners in Hope in Lilongwe, Malawi.

Funding

DGK is supported by Department of Internal Medicine, which is a part of the University of California, Los Angeles Health. NK is supported by the Fogarty International Center of the National Institutes of Health (NIH) under award number D43TW009343 and the University of California Global Health Institute (UCGHI). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or UCGHI.

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Figure.
India ink stain of cerebrospinal fluid for the described patient showing round, encapsulated organisms consistent with cryptococcal meningitis.