AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

MYCOLOGY

Evaluation of the Dynamiker Cryptococcal Antigen Lateral Flow Assay for the Diagnosis of HIV-Associated Cryptococcosis

Richard Kwizera,[a] Denis Omali,[a] Kiiza Tadeo,[a] John Kasibante,[a] Morris K. Rutakingirwa,[a] Enock Kagimu,[a] Kenneth Ssebambulidde,[a] Darlisha A. Williams,[a,b] Joshua Rhein,[a,b] David Boulware,[b] David B. Meya[a,b,c]

[a]Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda
[b]Division of Infectious Diseases and International Medicine, Department of Medicine, University of Minnesota, Minneapolis, Minnesota, USA
[c]Department of Medicine, School of Medicine, College of Health Sciences, Makerere University, Kampala, Uganda

ABSTRACT Cryptococcal meningitis is a leading cause of meningitis in sub-Saharan Africa. Given the need for rapid point-of-care testing, we evaluated the diagnostic performance of the Dynamiker cryptococcal antigen (CrAg) lateral flow assay (LFA). We assessed the diagnostic performance of the Dynamiker CrAg LFA compared to the IMMY CrAg LFA as the reference standard. We tested 150 serum, 115 plasma, and 100 cerebrospinal fluid (CSF) samples from HIV patients with symptomatic meningitis and 113 serum samples from patients with suspected asymptomatic cryptococcal antigenemia. Compared to the IMMY CrAg LFA, sensitivity of Dynamiker CrAg LFA was 98% in serum, 100% in plasma, 100% in CSF from symptomatic patients and 96% in serum from asymptomatic patients. Specificity was 66% in serum, 61% in plasma, and 91% in CSF from symptomatic patients, and 86% in serum from asymptomatic patients. The positive predictive value was 85% in serum, 82% in plasma, and 96% in CSF from symptomatic patients, and 69% in serum from asymptomatic patients. The negative predictive value was 94% in serum, 100% in plasma, and 100% in CSF from symptomatic patients, and 99% in serum from asymptomatic patients. The interassay reproducibility was 100% across the four sample types with no observed discordant results when Dynamiker CrAg LFA was tested in duplicate. However, a high number of false positives were observed on serum of symptomatic patients (11%), serum of asymptomatic patients (11%) and plasma of symptomatic patients (14%). The Dynamiker CrAg LFA had excellent sensitivity but poor specificity, particularly when tested on serum and plasma.

KEYWORDS cryptococcosis, diagnostic techniques, HIV, point-of-care systems, sensitivity and specificity

Cryptococcal meningitis (CM) is an opportunistic invasive fungal infection and the most common cause of adult meningitis in Africa (1). Cryptococcal meningitis accounts for approximately 15% of AIDS-related deaths (2–6). However, invasive pulmonary disease has been reported in non-AIDS patients (7). While other invasive fungal diseases go unrecognized in Uganda (8, 9), the index of clinical suspicion for cryptococcal meningitis is not low. Diagnosis of cryptococcal infection relies on detection of cryptococcal antigen (CrAg) in body fluids such as blood and cerebrospinal fluid (CSF). CSF culture is the historical standard for the diagnosis of cryptococcosis; however, CrAg tests clearly have superior sensitivity, particularly in early disease (10–12). India ink was traditionally used among people living with HIV/AIDS (13). However, India ink has a poor sensitivity that may lead to misdiagnosis, translating into high mortality rates in resource-limited settings where it is still being utilized to diagnose CM (12, 14). CrAg was also traditionally detected by latex agglutination tests and enzyme immunoassay (EIA) with more than 99% sensitivity (13, 15, 16) but with the major limitation of

Citation Kwizera R, Omali D, Tadeo K, Kasibante J, Rutakingirwa MK, Kagimu E, Ssebambulidde K, Williams DA, Rhein J, Boulware D, Meya DB. 2021. Evaluation of the Dynamiker cryptococcal antigen lateral flow assay for the diagnosis of HIV-associated cryptococcosis. J Clin Microbiol 59:e02421-20. https://doi.org/10.1128/JCM.02421-20.

Editor Kimberly E. Hanson, University of Utah

Address correspondence to Richard Kwizera, kwizerarichard@ymail.com.

Received 18 September 2020
Returned for modification 16 November 2020
Accepted 21 November 2020
Accepted manuscript posted online 2 December 2020
Published 18 February 2021

not being able to be performed at the point of care (POC) without laboratory facilities. However, more recently, detection of CrAg in whole blood, plasma, serum, and CSF using POC tests has revolutionized cryptococcal infection diagnosis with improved diagnostic performance and rapid turnaround time (10–12, 17).

Due to the increasing shift to POC testing, several companies have manufactured POC tests for detection of CrAg. Since 2011, the POC CrAg lateral flow assay (LFA) from Immuno-Mycologics, Inc. (IMMY; Norman, OK), has been shown to be more sensitive and user friendly for use with different sample types for the diagnosis if cryptococcosis than India ink, latex agglutination, and culture (10, 12, 17–21). Other CrAg LFAs include CryptoPS (Biosynex) and StrongStrep (Liming Bio), with suboptimal sensitivity and specificity, respectively (22, 23). The Dynamiker CrAg LFA (Dynamiker Biotechnology [Tianjin] Co., Ltd., China) is a dipstick sandwich immunochromatographic assay for the detection of capsular polysaccharide antigens of *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in human serum and CSF. However, there are limited data published about the diagnostic performance of the Dynamiker CrAg LFA. We therefore aimed to evaluate the diagnostic performance of the Dynamiker CrAg LFA for the diagnosis of HIV-associated cryptococcal disease in comparison to the FDA-approved IMMY CrAg LFA using different sample matrices.

MATERIALS AND METHODS

Study design and population. This was a retrospective diagnostic accuracy study, in which we evaluated the diagnostic performance of the Dynamiker CrAg LFA for the diagnosis of HIV-associated cryptococcal disease using anonymized samples from two prospective cohorts. The first cohort comprised cryopreserved samples from HIV patients with suspected cryptococcal antigenemia and CD4 counts of $<100\,\mu$l enrolled at the Infectious Diseases Institute (IDI) outpatient HIV clinic between November 2011 and May 2013 as a substudy of the Operational Research for Cryptococcal Antigen Screening (ORCAS) trial (ClinicalTrials.gov no. NCT01535469) (24). The second cohort included samples from consecutive symptomatic persons presenting in real time with suspected meningitis (e.g., fever of $\geq$38°C, headache, stiff neck, photophobia, altered mental status, and seizures) to Mulago National Referral Hospital in Kampala, Uganda, between November 2018 and September 2020, as a substudy of a meningitis screening study.

Participants included in both cohorts were $\geq$18 years old and HIV infected, with no prior cryptococcal disease. Participants in the first cohort were asymptomatic patients with suspected cryptococcal antigenemia, while the second cohort had symptomatic participants with suspected meningitis.

Ethics statement. Ethical approval for both studies was obtained from the Uganda National Council of Science and Technology (UNCST) and the Mulago Hospital Research and Ethics Committee. Patients or their caregivers provided written informed consent before study participation.

Study procedures. Using both the Dynamiker CrAg LFA and the IMMY CrAg LFA, we tested 150 serum, 115 plasma, and 100 CSF samples from patients with symptomatic meningitis and 113 serum samples from HIV-positive patients with CD4 counts of $<$100 cells/μl suspected of having and screened for asymptomatic cryptococcal antigenemia.

Procedure for IMMY cryptococcal antigen LFA test. The IMMY CrAg LFA kits were purchased from Immuno-Mycologics, Inc., Norman, OK. The IMMY CrAg LFA is a dipstick-type point-of-care lateral flow immunochromatographic assay that uses a test strip impregnated with gold-conjugated anticryptococcal monoclonal antibodies to detect cryptococcal capsular polysaccharide antigen (CrAg) of *Cryptococcus neoformans* and *Cryptococcus gattii*. The tests were performed according to the manufacturer's instructions, and results were read after 10 min at room temperature. Positive and negative controls were run for every set (1 to 10) of samples. For this procedure, specimens were diluted at a ratio of 1:1 in specimen diluent (40 μl of specimen and 40 μl of diluent). One trained laboratory technologist performed the tests, but two laboratory technologists read the test results to agree on the outcome.

Procedure for Dynamiker cryptococcal antigen LFA test. The Dynamiker LFA is also a dipstick sandwich immunochromatographic assay. The tests were performed according to the manufacturer's instructions, and the result was read after 15 min at room temperature. Positive and negative controls were run for every set (1 to 10) of samples. For this procedure, 80 μl of specimen was used without dilution. One trained laboratory technologist performed the tests, but two laboratory technologists read the test results to agree on the outcome. To test for interassay reproducibility, 67 samples were tested in duplicate before reaching saturation.

Statistical analysis. Data were analyzed using STATA version 13 (STATA, College Station, TX). Statistical analysis was aimed at comparing the sensitivity, specificity, positive predictive value, negative predictive value, area under the receiver operating characteristic curve (ROC-AUC), level of agreement, McNemar's test, and kappa test for reproducibility in the results of the Dynamiker CrAg LFA compared to the IMMY CrAg LFA as the reference standard.

TABLE 1 Summary of diagnostic performance of Dynamiker CrAg LFA using IMMY CrAg LFA as the reference standard

Patient population	Sample type (*n*)	No. (%) of:		% (95% CI)						
		False positives	False negatives	Sensitivity[b]	Specificity[c]	PPV[d]	NPV[e]	Agreement[f]	Kappa (*P*)	C statistic (95% CI)[a]
Symptomatic meningitis	Serum (150)	17 (11)	2 (1.3)	98 [98/100] (92.9–99.8)	66 [33/50] (51.2–78.8)	85.2 [98/115] (79.7–89.5)	94.3 [33/35] (80.5–98.5)	87.3 (80.9–92.2)	0.692 (<0.001)	0.820 (0.752–0.888)
	Plasma (115)	16 (14)	0 (0)	100 [74/74] (95.1–100)	60.9 [25/41] (44.5–75.8)	82.2 [74/90] (75.9–87.2)	100 [25/25] (86.3–100)	86.1 (78.4–91.8)	0.668 (<0.001)	0.805 (0.729–0.881)
	CSF (100)	3 (3)	0 (0)	100 [65/65] (94.5–100)	91.4 [32/35] (76.9–98.2)	95.6 [65/68] (88–98.5)	100 [32/32] (89.1–100)	97 (91.5–99.4)	0.933 (<0.001)	0.957 (0.910–1.000)
Suspected asymptomatic cryptococcal antigenemia	Serum (113)	12 (11)	1 (0.9)	96.4 [27/28] (81.7–99.9)	85.9 [73/85] (76.6–92.5)	69.2 [27/39] (57–79.3)	98.7 [73/74] (91.4–99.8)	88.5 (81.1–93.7)	0.727 (<0.001)	0.912 (0.861–0.963)

[a]Area under the ROC curve for sensitivity and specificity.
[b]Values in brackets are probability that a test result will be positive when the disease is present.
[c]Values in brackets are probability that a test result will be negative when the disease is not present.
[d]Values in brackets are probability that the disease is present when the test is positive.
[e]Values in brackets are probability that the disease is not present when the test is negative.
[f]Overall probability that a patient was correctly classified.

RESULTS

Diagnostic performance of the Dynamiker CrAg LFA compared to the IMMY CrAg LFA. For this retrospective laboratory-based interassay comparison study, we anonymized the samples to reduce bias, and therefore, baseline demographic characteristics of the population were not analyzed. However, demographics for the first cohort are published (24), while the study with cohort 2 is ongoing. Both tests were run concurrently for each sample.

Serum (symptomatic meningitis). For serum from 150 patients with symptomatic meningitis, the Dynamiker CrAg LFA had a sensitivity of 98% (98/100), specificity of 66% (33/50), positive predictive value (PPV) of 85.2% (98/115), and negative predictive value (NPV) of 94.3% (33/35). We registered 17 (11%) false positives and only 2 (1.3%) false negatives. There was an 87.3% agreement between the two tests using serum. There was statistical difference in performance (McNemar's test; $P < 0.001$). The area under the ROC curve for sensitivity and specificity showed moderate performance (C statistic = 0.820; 95% confidence interval [CI], 0.752 to 0.888) for the Dynamiker CrAg LFA (Table 1).

Plasma (symptomatic meningitis). For plasma samples from 115 patients with symptomatic meningitis, the Dynamiker LFA had a sensitivity of 100% (74/74), specificity of 60.9% (25/41), PPV of 82.2% (74/90), and NPV of 100% (25/25). We registered 16 (14%) false positives and no false negatives. There was an 86.1% agreement between the two tests using plasma (McNemar's test; $P < 0.001$). The area under the ROC curve for sensitivity and specificity showed moderate performance (C statistic = 0.805; 95% CI, 0.729 to 0.881) for the Dynamiker CrAg LFA.

CSF (symptomatic meningitis). For CSF samples from 100 patients with symptomatic meningitis, the Dynamiker LFA had a sensitivity of 100% (65/65), specificity of 91.4% (32/35), PPV of 95.6% (65/38), and NPV of 100% (32/32). We registered 3 (3%) false positives and no false negatives. There was a 97% agreement between the two tests using CSF (McNemar's test; $P = 0.12$). The area under the ROC curve for sensitivity and specificity showed excellent performance (C statistic = 0.957; 95% CI, 0.910 to 1.000) for the Dynamiker CrAg LFA.

Serum (suspected asymptomatic cryptococcal antigenemia). Using serum samples from 113 patients with CD4 counts of <100 cells/μl suspected of having and screened for asymptomatic cryptococcal antigenemia, the Dynamiker LFA had a sensitivity of 96.4% (27/28), specificity of 85.9% (73/85), PPV of 69.2% (27/39), and NPV of 98.7% (73/74). We registered 12 (11%) false positives and only 1 (0.9%) false negative. There was an 88.5% agreement between the two tests using serum from asymptomatic patients (McNemar's test; $P = 0.003$). The area under the receiver-operator characteristic (ROC) curve for sensitivity and specificity showed moderate performance (C statistic = 0.912; 95% CI, 0.861 to 0.963) for the Dynamiker CrAg LFA.

The interassay reproducibility was 100% across the four groups with no observed

discordant results when Dynamiker CrAg LFA was tested in duplicate ($n = 67$; kappa $P < 0.001$).

DISCUSSION

This study determined the diagnostic performance of the Dynamiker CrAg LFA in two populations/cohorts, i.e., (i) HIV patients with suspected cryptococcal antigenemia and CD4 counts of $<100\ \mu$l and (ii) HIV patients with symptomatic meningitis using serum, plasma, and CSF. Overall, the Dynamiker assay had excellent sensitivity, but the specificity was poor, particularly in serum or plasma. The Dynamiker CrAg LFA registered a high number of false positives in serum and plasma of symptomatic patients. In screenings of persons with CD4 counts of <100 cells/μl with an expected 6% CrAg prevalence, the positive predictive value of a positive test in serum or plasma would be 30% or less; however, in our study, the PPV was $>60\%$ (6).

When samples were tested, the Dynamiker CrAg LFA test strips often displayed stronger bands, while the IMMY CrAg LFA displayed very weak bands. Similarly, at presumed low antigen concentrations, the Dynamiker CrAg LFA test strips displayed weak positive bands while the IMMY CrAg LFA strips were negative. This is reflected by the stated Dynamiker CrAg LFA limit of detection (LOD) of 1.25 ng/ml, which is lower than the IMMY CrAg LFA LOD of 1.75 ng/ml. This "extra sensitivity" could explain the high number of false positives observed in serum and plasma samples. Based on this observation, it is clear that the Dynamiker CrAg LFA has a sensitivity comparable to that of IMMY CrAg LFA for all sample types but has poor specificity, particularly in serum and plasma samples. It is possible that the Dynamiker CrAg LFA might be a better test than IMMY CrAg LFA due to its lower LOD, and the high number of false positives might actually be true positives. It might be wise in the future to evaluate the two tests against a common reference standard, like culture or PCR. However, a recent poster from the 28th ECCMID also indicated that band intensity was in general stronger for the Dynamiker CrAg LFA, in light of which the authors (not the manufacturer) recommended that a weakly positive test result (1+) be regarded as negative in order not to compromise specificity (25).

From a laboratory perspective, the Dynamiker CrAg LFA had two practical advantages. First, it did not require diluting the sample, although this may be partially responsible for problems with specificity. Second, the Dynamiker CrAg LFA kits are individually packaged, which prevents any form of deterioration or possible contamination, such as may occur when a container is opened multiple times to extract a strip whenever a CrAg test needs to be done. However, downsides of individual packaging are higher shipping costs and greater storage space required.

The main limitation to the study was that we were unable to correlate our results to other laboratory tests (e.g., culture) or clinical data. Second, the two tests have different limits of detection, which could explain the observed false-positive results. Despite these limitations, this study demonstrated acceptable sensitivity for the Dynamiker CrAg LFA with different sample matrices but poor specificity when serum and plasma samples are used. Further refinements are needed to improve the specificity for this assay to have acceptable performance for use.

ACKNOWLEDGMENTS

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. Support for the parent study was from National Institute of Neurologic Disorders and Stroke (R01NS086312), the Fogarty International Center (K01TW010268, J.R.). R.K. and D.B.M. are supported through DELTAS Africa Initiative grant no. DEL-15-011 to THRiVE-2, Wellcome Trust grant no. 107742/Z/15/Z, and the United Kingdom government.

We are grateful for institutional support from the Infectious Diseases Institute. We also thank the entire meningitis team for patient care. The Dynamiker CrAg LFA kits were provided freely by Dynamiker Biotechnology (Tianjin) Co., Ltd.

We declare no conflict of interest. The manufacturers of the diagnostic tests had no role in study design, data analysis, or writing of the manuscript.

R.K. and D.B.M. conceived and designed the protocol. R.K. and D.O. performed laboratory tests. K.T. collected samples. J.K., M.K.R., E.K., and K.S. performed patient care. R.K. analyzed data. R.K. participated in initial manuscript drafting. R.K., D.O., K.T., J.K., M.K.R., E.K., K.S., D.A.W., J.R., D.B., and D.B.M. participated in critical revisions for intellectual content. D.A.W., J.R., D.B., and D.B.M. participated in administrative, technical, or material support.

REFERENCES

1. Durski KN, Kuntz KM, Yasukawa K, Virnig BA, Meya DB, Boulware DR. 2013. Cost-effective diagnostic checklists for meningitis in resource-limited settings. J Acquir Immune Defic Syndr 63:e101–e108. https://doi.org/10.1097/QAI.0b013e31828e1e56.
2. French N, Gray K, Watera C, Nakiyingi J, Lugada E, Moore M, Lalloo D, Whitworth JA, Gilks CF. 2002. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. AIDS 16:1031–1038. https://doi.org/10.1097/00002030-200205030-00009.
3. Liechty CA, Solberg P, Were W, Ekwaru JP, Ransom RL, Weidle PJ, Downing R, Coutinho A, Mermin J. 2007. Asymptomatic serum cryptococcal antigenemia and early mortality during antiretroviral therapy in rural Uganda. Trop Med Int Health 12:929–935. https://doi.org/10.1111/j.1365-3156.2007.01874.x.
4. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. 2009. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS 23:525–530. https://doi.org/10.1097/QAD.0b013e328322ffac.
5. Parkes-Ratanshi R, Achan B, Kwizera R, Kambugu A, Meya D, Denning DW. 2015. Cryptococcal disease and the burden of other fungal diseases in Uganda; Where are the knowledge gaps and how can we fill them? Mycoses 58(Suppl 5):85–93. https://doi.org/10.1111/myc.12387.
6. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, Denning DW, Loyse A, Boulware DR. 2017. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis 17:873–881. https://doi.org/10.1016/S1473-3099(17)30243-8.
7. Kalyango N, Kwizera R, Baluku JB, Bongomin F. 2020. Intra-cavitary pulmonary cryptococcoma in poorly controlled diabetes mellitus. Med Mycol Case Rep 28:1–3. https://doi.org/10.1016/j.mmcr.2020.02.005.
8. Kwizera R, Bongomin F, Lukande R. 2020. Deep fungal infections diagnosed by histology in Uganda: a 70-year retrospective study. Med Mycol 58:1044–1052. https://doi.org/10.1093/mmy/myaa018.
9. Kwizera R, Bongomin F, Meya DB, Denning DW, Fahal AH, Lukande R. 2020. Mycetoma in Uganda: a neglected tropical disease. PLoS Negl Trop Dis 14:e0008240. https://doi.org/10.1371/journal.pntd.0008240.
10. Williams DA, Kiiza T, Kwizera R, Kiggundu R, Velamakanni S, Meya DB, Rhein J, Boulware DR. 2015. Evaluation of fingerstick cryptococcal antigen lateral flow assay in HIV-infected persons: a diagnostic accuracy study. Clin Infect Dis 61:464–467. https://doi.org/10.1093/cid/civ263.
11. Ssebambulidde K, Bangdiwala AS, Kwizera R, Kandole TK, Tugume L, Kiggundu R, Mpoza E, Nuwagira E, Williams DA, Lofgren SM, Abassi M, Musubire AK, Cresswell FV, Rhein J, Muzoora C, Hullsiek KH, Boulware DR, Meya DB, Adjunctive Sertraline for Treatment of HIVaCMT. 2019. Symptomatic cryptococcal antigenemia presenting as early cryptococcal meningitis with negative cerebral spinal fluid analysis. Clin Infect Dis 68:2094–2098. https://doi.org/10.1093/cid/ciy817.
12. Boulware DR, Rolfes MA, Rajasingham R, von Hohenberg M, Qin Z, Taseera K, Schutz C, Kwizera R, Butler EK, Meintjes G, Muzoora C, Bischof JC, Meya DB. 2014. Multisite validation of cryptococcal antigen lateral flow assay and quantification by laser thermal contrast. Emerg Infect Dis 20:45–53. https://doi.org/10.3201/eid2001.130906.
13. Bicanic T, Harrison TS. 2004. Cryptococcal meningitis. Br Med Bull 72:99–118. https://doi.org/10.1093/bmb/ldh043.
14. Satishchandra P, Mathew T, Gadre G, Nagarathna S, Chandramukhi A, Mahadevan A, Shankar SK. 2007. Cryptococcal meningitis: clinical, diagnostic and therapeutic overviews. Neurol India 55:226–232. https://doi.org/10.4103/0028-3886.35683.
15. Kambugu A, Meya DB, Rhein J, O'Brien M, Janoff EN, Ronald AR, Kamya MR, Mayanja-Kizza H, Sande MA, Bohjanen PR, Boulware DR. 2008. Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. Clin Infect Dis 46:1694–1701. https://doi.org/10.1086/587667.
16. Binnicker MJ, Jespersen DJ, Bestrom JE, Rollins LO. 2012. Comparison of four assays for the detection of cryptococcal antigen. Clin Vaccine Immunol 19:1988–1990. https://doi.org/10.1128/CVI.00446-12.
17. Rolfes M, Butler E, von Hohenberg M, Nabeta H, Kwizera R, Rajasingham R, Bahr N, Bohjanen P, Meya D, Boulware D. 2012. Evaluation of a novel point-of-care lateral flow assay to detect cryptococcal antigen in plasma and CSF, poster 953. *In* 19th Conference on Retroviruses and Opportunistic Infections, Seattle, WA, 5 to 8 March 2012.
18. Hansen J, Slechta ES, Gates-Hollingsworth MA, Neary B, Barker AP, Bauman S, Kozel TR, Hanson KE. 2013. Large-scale evaluation of the immuno-mycologics lateral flow and enzyme-linked immunoassays for detection of cryptococcal antigen in serum and cerebrospinal fluid. Clin Vaccine Immunol 20:52–55. https://doi.org/10.1128/CVI.00536-12.
19. Drain PK, Hong T, Krows M, Govere S, Thulare H, Wallis CL, Gosnell BI, Moosa MY, Bassett IV, Celum C. 2019. Validation of clinic-based cryptococcal antigen lateral flow assay screening in HIV-infected adults in South Africa. Sci Rep 9:2687. https://doi.org/10.1038/s41598-018-37478-7.
20. Jarvis JN, Percival A, Bauman S, Pelfrey J, Meintjes G, Williams GN, Longley N, Harrison TS, Kozel TR. 2011. Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. Clin Infect Dis 53:1019–1023. https://doi.org/10.1093/cid/cir613.
21. Lindsley MD, Mekha N, Baggett HC, Surinthong Y, Autthateinchai R, Sawatwong P, Harris JR, Park BJ, Chiller T, Balajee SA, Poonwan N. 2011. Evaluation of a newly developed lateral flow immunoassay for the diagnosis of cryptococcosis. Clin Infect Dis 53:321–325. https://doi.org/10.1093/cid/cir379.
22. Mpoza E, Mukaremera L, Kundura DA, Akampurira A, Luggya T, Tadeo KK, Pastick KA, Bridge SC, Tugume L, Kiggundu R, Musubire AK, Williams DA, Muzoora C, Nalintya E, Rajasingham R, Rhein J, Boulware DR, Meya DB, Abassi M. 2018. Evaluation of a point-of-care immunoassay test kit 'StrongStep' for cryptococcal antigen detection. PLoS One 13:e0190652. https://doi.org/10.1371/journal.pone.0190652.
23. Skipper C, Tadeo K, Martyn E, Nalintya E, Rajasingham R, Meya DB, Kafufu B, Rhein J, Boulware DR. 2020. Evaluation of serum cryptococcal antigen testing using two novel semiquantitative lateral flow assays in persons with cryptococcal antigenemia. J Clin Microbiol 58:e02046-19. https://doi.org/10.1128/JCM.02046-19.
24. Meya DB, Kiragga AN, Nalintya E, Morawski BM, Rajasingham R, Park BJ, Mubiru A, Kaplan JE, Manabe YC, Boulware DR. 2019. Reflexive laboratory-based cryptococcal antigen screening and preemptive fluconazole therapy for cryptococcal antigenemia in HIV-infected individuals with CD4 $<$100 cells/μL: a stepped-wedge, cluster-randomized trial. J Acquir Immune Defic Syndr 80:182–189. https://doi.org/10.1097/QAI.0000000000001894.
25. Björnsdóttir MK, Mahler SS, Raluca D, Arendrup MC. 2019. Evaluation of the new Dynamiker cryptococcal antigen lateral flow assay (LFA) in comparison with IMMY LFA and Meridian latex agglutination test, poster 1197. *In* 28th ECCMID, Madrid, Spain, 21 to 24 April 2018.