Neglecting Genetic Diversity Hinders Timely Diagnosis of *Cryptococcus* Infections

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More than a decade ago, the first cryptococcal lateral flow assay (LFA) became available. This affordable point-of-care test has undeniably led to tremendous improvements in early detection of cryptococcosis (1, 2). However, from several studies, it must be concluded that the reliability of recently introduced assays falls short, thereby negatively impacting timely detection and thus hindering an effective treatment of cryptococcal infections (3–6). The apparent reduced performance of newly commercialized cryptococcal LFAs might be caused by ignoring the genetic diversity as manufacturers focus on the four serotypes A to D. Whereas the first commercially available cryptococcal LFA (IMMY Diagnostics, Norman, OK, USA) was extensively evaluated (1, 7), this does not seem to be the case with LFAs from other manufacturers in that their product information indicates that only one strain per serotype was used for product development. The observation of neglecting the genetic diversity within the *Cryptococcus gattii*/*Cryptococcus neoformans* species complexes, combined with reports of false-negative LFAs not related to the pro-/postzone effect (8–10), inspired us to compare commercially available LFAs with a set of well-defined strains that reflects the genetic diversity within the *C. gattii*/*C. neoformans* species complexes.

Four Conformité Européenne (CE)/*in vitro* diagnostic (IVD)-marked LFAs were tested: cryptococcal antigen LFA (IMMY Diagnostics), CryptoPS (Biosynex, Illkirch-Graffenstaden, France), cryptococcal antigen LFA (Dynamiker Biotechnology, Tianjin, China), and cryptococcal capsular polysaccharide detection K-set (FungiXpert, Genobio Pharmaceutical, Tianjin, China). Forty cryptococcal strains comprising all seven recognized species and their interspecies hybrids were tested (Table 1) (11). A loop full of freshly grown *Cryptococcus* was suspended into glucose-yeast extract-peptone broth and incubated at 35°C for 16 h, and the homogenized culture was tested according to the manufacturer's instructions. When a strain tested negative, 10-, 100-, and 1,000-fold dilutions were retested to exclude the postzone effect (10).

All seven pathogenic *Cryptococcus* species were detected by the IMMY Diagnostics and FungiXpert LFAs. *Cryptococcus bacillisporus* and two *Cryptococcus tetragattii* strains, all three serotype C, could not be detected with the Dynamiker LFA (Table 1). The Biosynex LFA could not detect one *C. bacillisporus* (serotype C) and one *C. deuterogattii* (serotype B) strain and none of the six *C. tetragattii* (serotype C) strains (Table 1).

This indicates that for two LFAs in particular, *C. tetragattii* is a blind spot, a worrisome false-negative result because *C. tetragattii* infections are common in sub-Saharan Africa and the Indian subcontinent, regions with the highest number of HIV-associated

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TABLE 1 *Cryptococcus* species and their detection by four CE/IVD cryptococcal antigen lateral flow assays

Cryptococcal species and genotype	Detection (n [%]) with:			
	IMMY Diagnostics	FungiXpert	Dynamiker	Biosynex
<i>Cryptococcus neoformans</i> , serotype A; genotypes AFLP1/VNI, AFLP1A/VNB/VNII, AFLP1B/VNII	7/7 (100)	7/7 (100)	7/7 (100)	7/7 (100)
<i>Cryptococcus deneoformans</i> , serotype D; genotype AFLP2/VNIV	6/6 (100)	6/6 (100)	6/6 (100)	6/6 (100)
<i>Cryptococcus gattii sensu stricto</i> , serotype B; genotype AFLP4/VGI	4/4 (100)	4/4 (100)	4/4 (100)	4/4 (100)
<i>Cryptococcus bacillisporus</i> , serotype B and C; genotype AFLP5/VGIII	4/4 (100)	4/4 (100)	3/4 (75)^a	3/4 (75)^a
<i>Cryptococcus deuterogattii</i> , serotype B; genotype AFLP6/VGII	5/5 (100)	5/5 (100)	5/5 (100)	4/5 (80)
<i>Cryptococcus tetragattii</i> , serotype C; genotype AFLP7/VGIV	6/6 (100)	6/6 (100)	4/6 (66.6)	0/6 (0)
<i>Cryptococcus decagattii</i> , serotype B; genotype AFLP10	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)
<i>Cryptococcus deneoformans</i> × <i>Cryptococcus neoformans</i> hybrid, serotype AD; genotype AFLP3/VNIII	4/4 (100)	4/4 (100)	4/4 (100)	4/4 (100)
<i>Cryptococcus deneoformans</i> × <i>Cryptococcus gattii</i> hybrid, serotype BD; genotype AFLP8	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)
<i>Cryptococcus gattii</i> × <i>Cryptococcus neoformans</i> interspecies, serotype AB; genotype AFLP9	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)

^aThe Dynamiker and Biosynex LFAs could not detect a serotype C strain of *C. bacillisporus*. The boldfaced values indicate the presence of false-negative test results.

cryptococcal meningitis cases (2, 11–13). Importantly, *C. tetragattii* was involved in 13% to 20% of the HIV-associated cryptococcal meningitis cases (13, 14). The Biosynex LFA was recently used in studies conducted in Botswana (6) and Uganda (3); thereby, the reported false-negative samples hinted strongly at the presence of *C. tetragattii* isolates among the clinical specimens. We therefore argue that studies involving diagnostic assay setup and/or testing consider the (local) epidemiology, genetic background, and taxonomic diversity of the involved pathogen/species complex. Ultimately, the dogma that the genetic diversity of pathogenic *Cryptococcus* is covered by single representatives of each of the serotypes A to D is outdated (11, 15). Consideration of the revised cryptococcal taxonomy in the product setup and validation may alleviate the loss of sensitivity due to false negatives.

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REFERENCES

- 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>.
- Rajasingham R, Wake RM, Beyene T, Katende A, Letang E, Boulware DR. 2018. Cryptococcal meningitis diagnostics and screening in the era of point-of-care laboratory testing. *J Clin Microbiol* 57:e01238–18. <https://doi.org/10.1128/JCM.01238-18>.
- 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>.
- Tansarli GS, Chapin KC. 2020. Diagnostic test accuracy of the BioFire FilmArray meningitis/encephalitis panel: a systematic review and meta-analysis. *Clin Microbiol Infect* 26:281–290. <https://doi.org/10.1016/j.cmi.2019.11.016>.
- Temfack E, Rim JJB, Spijker R, Loyse A, Chiller T, Pappas PG, Perfect J, Sorell TC, Harrison TS, Cohen JF, Lortholary O. 2020. Cryptococcal antigen in serum and cerebrospinal fluid for detecting cryptococcal meningitis in adults living with HIV: systematic review and meta-analysis of diagnostic test accuracy studies. *Clin Infect Dis*:ciaa1243. <https://doi.org/10.1093/cid/ciaa1243>.
- Tenforde MW, Boyer-Chammard T, Muthoga C, Tawe L, Milton T, Rulaganyang I, Lechiile K, Rukasha I, Leeme TB, Govender NP, Ngidi J, Mine M, Molloy SF, Harrison TS, Lortholary O, Jarvis JN. 2020. Diagnostic accuracy of the Biosynex CryptoPS cryptococcal antigen semi-quantitative lateral flow assay in patients with advanced HIV disease. *J Clin Microbiol* 59:e02307–20. <https://doi.org/10.1128/JCM.02307-20>.
- 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>.
- Eshwara VK, Garg R, Chandrashekar GS, Shaw T, Mukhopadhyay C. 2018. Fatal *Cryptococcus gattii* meningitis with negative cryptococcal antigen test in a HIV-non-infected patient. *Indian J Med Microbiol* 36:439–440. https://doi.org/10.4103/ijmm.JMM_18_164.
- Navabi N, Montebatsi M, Scott M, Gluckman SJ, Reid MJ. 2015. Case report: false negative serum cryptococcal latex agglutination test in a patient with disseminated cryptococcal disease. *J Int Assoc Provid AIDS Care* 14:123–126. <https://doi.org/10.1177/2325957414555233>.
- Stewart TW, Parnell D. 1982. Postzone v prozone. *JAMA* 248:646–647. <https://doi.org/10.1001/jama.1982.03330060014011>.
- Hagen F, Khayhan K, Theelen B, Kolecka A, Polacheck I, Sionov E, Falk R, Parnmen S, Lumsch HT, Boekhout T. 2015. Recognition of seven species in the *Cryptococcus gattii*/*Cryptococcus neoformans* species complex. *Fungal Genet Biol* 78:16–48. <https://doi.org/10.1016/j.fgb.2015.02.009>.
- Lahiri Mukhopadhyay S, Bahubali VH, Manjunath N, Swaminathan A, Maji S, Palaniappan M, Parthasarathy S, Chandrashekar N. 2017. Central nervous system infection due to *Cryptococcus gattii sensu lato* in India: analysis of clinical features, molecular profile and antifungal susceptibility. *Mycoses* 60:749–757. <https://doi.org/10.1111/myc.12656>.
- Nyazika TK, Hagen F, Meis JF, Robertson VJ. 2016. *Cryptococcus tetragattii* as a major cause of cryptococcal meningitis among HIV-infected

- individuals in Harare, Zimbabwe. *J Infect* 72:745–752. <https://doi.org/10.1016/j.jinf.2016.02.018>.
14. Litvintseva AP, Thakur R, Reller LB, Mitchell TG. 2005. Prevalence of clinical isolates of *Cryptococcus gattii* serotype C among patients with AIDS in sub-Saharan Africa. *J Infect Dis* 192:888–892. <https://doi.org/10.1086/432486>.
15. Hagen F, Lumbsch HT, Arsic Arsenijevic V, Badali H, Bertout S, Billmyre RB, Bragulat MR, Cabañes FJ, Carbia M, Chakrabarti A, Chaturvedi S, Chaturvedi V, Chen M, Chowdhary A, Colom MF, Cornely OA, Crous PW, Cuétara MS, Diaz MR, Espinel-Ingroff A, Fakhim H, Falk R, Fang W, Herkert PF, Ferrer Rodríguez C, Fraser JA, Gené J, Guarro J, Idnurm A, Illnait-Zaragozi MT, Khan Z, Khayhan K, Kolečka A, Kurtzman CP, Lagrou K, Liao W, Linares C, Meis JF, Nielsen K, Nyazika TK, Pan W, Pekmezovic M, Polacheck I, Posteraro B, de Queiroz Telles F, Romeo O, Sánchez M, Sampaio A, Sanguinetti M, Sriburee P, Sugita T, Taj-Aldeen SJ, Takashima M, Taylor JW, Theelen B, Tomazin R, Verweij PE, Wahyuningsih R, Wang P, Boekhout T. 2017. Importance of resolving fungal nomenclature: the case of multiple pathogenic species in the *Cryptococcus* genus. *mSphere* 2:e00238-17. <https://doi.org/10.1128/mSphere.00238-17>.