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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
Cryptococcus neoformans, serotype A; genotypes AFLP1/VNI, AFLP1A/VNB/VNII, AFLP1B/VNII	7/7 (100)	7/7 (100)	7/7 (100)	7/7 (100)
Cryptococcus deneoformans , serotype D; genotype AFLP2/VNIV	6/6 (100)	6/6 (100)	6/6 (100)	6/6 (100)
Cryptococcus gattii sensu stricto, serotype B; genotype AFLP4/VGI	4/4 (100)	4/4 (100)	4/4 (100)	4/4 (100)
Cryptococcus bacillisporus, serotype B and C; genotype AFLP5/VGIII	4/4 (100)	4/4 (100)	**3/4 (75)**[a]	**3/4 (75)**[a]
Cryptococcus deuterogattii, serotype B; genotype AFLP6/VGII	5/5 (100)	5/5 (100)	5/5 (100)	**4/5 (80)**
Cryptococcus tetragattii, serotype C; genotype AFLP7/VGIV	6/6 (100)	6/6 (100)	**4/6 (66.6)**	**0/6 (0)**
Cryptococcus decagattii, serotype B; genotype AFLP10	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)
Cryptococcus deneoformans × *Cryptococcus neoformans* hybrid, serotype AD; genotype AFLP3/VNIII	4/4 (100)	4/4 (100)	4/4 (100)	4/4 (100)
Cryptococcus deneoformans × *Cryptococcus gattii* hybrid, serotype BD; genotype AFLP8	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)
Cryptococcus gattii × *Cryptococcus neoformans* interspecies, serotype AB; genotype AFLP9	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)

[a]The 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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