

# Large-Scale Evaluation of the Immuno-Mycologics Lateral Flow and Enzyme-Linked Immunoassays for Detection of Cryptococcal Antigen in Serum and Cerebrospinal Fluid

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**Cryptococcosis is a systemic infection caused by the pathogenic yeasts *Cryptococcus neoformans* and *C. gattii*. Detection of cryptococcal capsular antigen (CrAg) in serum and cerebrospinal fluid (CSF) plays an important diagnostic role. We prospectively compared the new Immuno-Mycologics Inc. (IMMY) lateral flow assay (LFA) and enzyme immunoassay (EIA) to our current CrAg test (Premier EIA; Meridian Bioscience Inc.). Discordant samples were retested with the latex-*Cryptococcus* antigen test (IMMY) and using serotype-specific monoclonal antibodies (MAbs). A total of 589 serum and 411 CSF specimens were tested in parallel. Qualitative agreement across assays was 97.7%. In all, 56 (41 serum and 15 CSF) samples were positive and 921 (527 serum and 394 CSF) samples were negative by all three assays. The 23 discrepant specimens were all Meridian EIA negative. Of 23 discordant specimens, 20 (87.0%) were positive by both the IMMY LFA and EIA, 2 were LFA positive only, and 1 was EIA positive only. Eleven discrepant specimens had adequate volume for latex agglutination (LA) testing; 8 were LA positive, and 3 were LA negative. LA-negative samples (2 CSF samples and 1 serum) had low IMMY LFA/EIA titers ( $\leq 1:10$ ). Serotype-specific MAb analysis of the LA-positive samples suggested that these specimens contained CrAg epitopes similar to those of serotype C strains. In conclusion, the IMMY assays showed excellent overall concordance with the Meridian EIA. Assay performance differences were related to issues of analytic sensitivity and possible serotype bias. Incomplete access to patient-level data combined with low specimen volumes limited our ability to fully resolve discrepant results.**

*Cryptococcus* spp. are encapsulated, yeast-like fungi that exist as saprobes in nature. Cryptococcosis, an invasive disease caused primarily by the pathogenic species *Cryptococcus neoformans* and *C. gattii*, is one of the most important opportunistic infections affecting immunocompromised patients worldwide. Immunoassays for the detection of cryptococcal capsular polysaccharide antigen (CrAg) in serum and cerebrospinal fluid (CSF) have played an integral role in the diagnosis of invasive disease since the first description of a latex agglutination assay nearly 50 years ago (1).

A variety of different immunoassays are cleared by the U.S. Food and Drug Administration (FDA) for the diagnosis of cryptococcosis. These assays include latex agglutination (LA)-based tests, antigen capture sandwich enzyme immunoassays (EIAs), and a lateral flow immunochromatographic assay (LFA) (2–4). The antigen target for all tests is glucuronoxylomannan (GXM), the primary polysaccharide component of the cryptococcal capsule. GXM occurs as four major serotypes—A, B, C, and D—and a hybrid serotype, AD (5, 6). Serotypes A and D make up the large majority of *C. neoformans* clinical isolates. Serotype B and C isolates are classified as *C. gattii* based on biochemical and molecular genetic features that differentiate them from serotype A and D isolates (7).

The sensitivities of four commercially available CrAg immunoassays were recently evaluated using purified GXM isolated from serotype A, B, C, and D strains (8). Several of the assays tested, including the kit currently used in our laboratory, showed reduced sensitivity for serotype C GXM (8). The purpose of this study was to evaluate a new CrAg LFA and EIA (Immuno-Mycologics Inc. [IMMY], Norman, OK) in comparison to our current EIA (Meridian Bioscience Inc., Cincinnati, OH). We also sought

to determine whether serotype bias influences assay test performance by using a large number of serum and CSF specimens and anti-GXM monoclonal antibodies (MAbs) with differing reactivities toward each of the major cryptococcal serotypes.

(This study was presented in part at the 112th General Meeting of the American Society for Microbiology, San Francisco, CA.)

## MATERIALS AND METHODS

Serum and CSF specimens submitted to ARUP Laboratories for CrAg testing between May and November 2011 were included in the analysis. Specimens with sufficient volume were tested in parallel using the IMMY CrAg LFA, IMMY Alpha EIA, and Meridian Premier EIA per the manufacturers' instructions. Laboratory records were reviewed to confirm the specimen type and the geographic location (state) of the patient. Qualitative test results and endpoint titers ( $\pm 1$  dilution) were compared with the percent agreement and the kappa statistic. Measures of agreement by the kappa statistic were categorized as near perfect (0.8 to 0.99), substantial (0.61 to 0.8), moderate (0.41 to 0.6), fair (0.21 to 0.4), slight (0.01 to 0.2), or poor (0). Differences in proportions were assessed with the Fisher exact test or the chi-square test. Statistical analyses were performed using Analyze-it software, version 2.26 (Leeds, United Kingdom). Indeterminate

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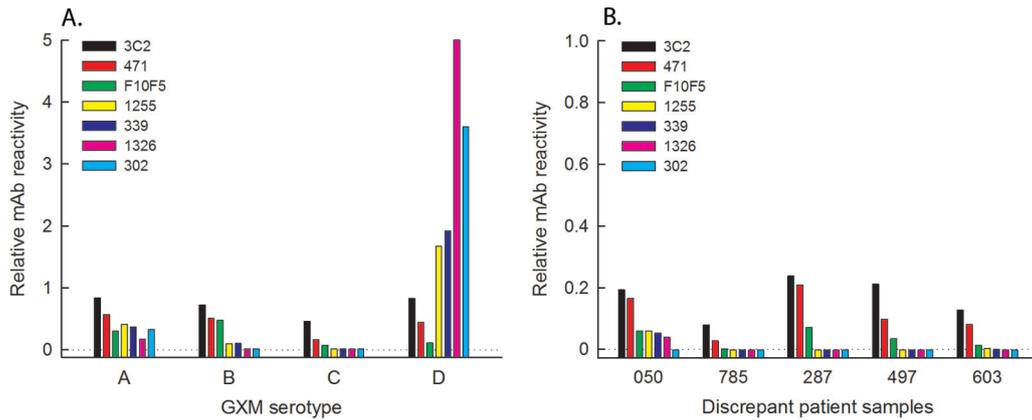
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**FIG 1** MAb binding patterns for purified GXM (A) and discordant patient specimens (B). Refer to Materials and Methods for a detailed description of the anti-GXM EIA. MAb, monoclonal antibody; GXM, glucuronoxylomannan.

CrAg results were considered to be negative for the comparison studies. The study was approved by the University of Utah Institutional Review Board (IRB).

**IMMY LFA.** The IMMY LFA is a dipstick sandwich immunochromatographic assay that utilizes specimen wicking to capture gold-conjugated, anti-*Cryptococcus* MAbs deposited on the test membrane. An optimized mixture of two anti-GXM MAbs, F12D2 and 339, is used to capture and then detect CrAg (3). No reagent preparation is required. Test results are read after 10 min, as the presence or absence of a positive-control line with or without a visible specimen test line. In our study, LFA titers were then determined by diluting patient samples in diluent and assessing the reactivity of the control and specimen lines. A single technologist performed and interpreted all of the LFA results for this study. An evaluation of clinical test characteristics was previously reported, using serum and urine specimens collected from HIV-positive patients (3, 9).

**IMMY EIA.** The Alpha EIA is a direct, microplate-based, immunoenzymatic sandwich assay. The test protocol includes wash buffer and diluent preparation, 3 incubation steps (two for 30 min and one for 10 min), and 6 washes. The same mixtures of capture and detection MAbs that are utilized in the LFA strips are combined in this EIA format. In the EIA, however, the detection MAbs are conjugated to horseradish peroxidase. Qualitative test results were determined using a spectrophotometer set at wavelengths of 450 nm and 630 nm. A positive result was defined as an optical density at 450/630 nm ( $OD_{450/630}$ ) of  $>0.265$ , and a negative result was defined as an  $OD_{450/630}$  of  $\leq 0.265$ . Positive specimens were serially diluted and the EIA titer calculated using an equation based on the OD of the blank and a multiplication factor.

**Meridian EIA.** The Meridian Premier EIA utilizes an anti-*Cryptococcus* polyclonal capture antibody adsorbed to microwell plates in combination with a MAb-peroxidase conjugate. Similar to the IMMY EIA, performance of the Meridian assay requires preparation of a wash buffer, 3 incubation steps (10 min each), and 8 washes. Qualitative results are also read on a dual-wavelength plate reader set at 450/630 nm. The following test cutoffs were applied per the instructions in the package insert: negative result,  $OD_{450/630}$  of  $<0.070$ ; indeterminate result,  $OD_{450/630}$  of  $\geq 0.070$  to  $<0.100$ ; and positive result,  $OD_{450/630}$  of  $\geq 0.100$ . Positive specimens were serially diluted and the EIA titer calculated using an equation based on the OD of the blank and a multiplication factor.

**Discrepancy testing.** Specimens displaying discordant results were reanalyzed with the latex-*Cryptococcus* antigen test (IMMY), a polyclonal LA test shown to detect serotypes A, B, C, and D (8). The manufacturer’s instructions were modified slightly to accommodate repeat testing of low-volume specimens. Briefly, the specimen volume was reduced from 300  $\mu$ l to 150  $\mu$ l, and the serum pronase treatment was also cut by half.

In an attempt to determine the GXM epitopes present in discordant specimens, samples with adequate volume were reanalyzed using a panel

of MAbs known to have different serotype recognition patterns (Fig. 1A). The antibody panel included three MAbs that recognize serotypes A, B, C, and D (F12D2, 3C2, and 471), one that detects serotypes A and B (F10F5), two that react with serotypes A, B, and D (1255 and 339), and two directed against serotypes A and D (1326 and 302) (10–15). GXM EIAs were performed as previously described (8), with the following modifications. Plates were coated overnight with a 1:1 combination of capture MAbs F12D2 and 339. Discrepant specimens and purified GXM were serially diluted across plates in phosphate-buffered saline (PBS) containing 0.05% Tween. Bound polysaccharide was detected by incubation with each of eight horseradish peroxidase-conjugated MAbs followed by a peroxidase substrate. Assays were completed by adding stop solution, and results were read at 450 nm. The log OD was plotted against the log titer or log concentration (ng/ml) and fit to a linear regression with correction for background, and the titer/concentration that produced an OD of 0.5 was calculated from the regression and taken as the endpoint. F12D2, a MAb that has strong reactivity with all four serotypes, was used to normalize endpoints to account for differing GXM concentrations in clinical specimens. Normalized values were calculated as the quotient of each MAB endpoint (ng/ml) divided by the F12D2 endpoint (ng/ml) and are reported as “relative MAb reactivities” for discrepant samples and GXMs.

**RESULTS**

Over the 5-month study period, 1,000 specimens (589 serum and 411 CSF specimens) were tested in parallel. The LFA required the fewest steps and the least hands-on time to perform, but the interpretation of results was more subjective than that of the spectrophotometric methods.

**Assay comparisons.** Summaries of the test results are presented in Tables 1 and 2. In all, 56 (41 serum and 15 CSF) speci-

**TABLE 1** Qualitative test results

IMMY test result	No. of samples with Meridian EIA result			
	CSF		Serum	
	Positive	Negative	Positive	Negative
IMMY LFA results				
Positive	15	1	41	21
Negative	0	395	0	527
IMMY EIA results				
Positive	15	2	41	19
Negative	0	394	0	529

TABLE 2 Qualitative assay agreement

Comparison and agreement	% Agreement	Kappa value (95% CI)
Meridian EIA vs IMMY LFA	97.8	0.82 (0.75–0.9)
Positive agreement	71.8	
Negative agreement	97.7	
Meridian EIA vs IMMY EIA	98.0	0.84 (0.77–0.91)
Positive agreement	73.7	
Negative agreement	97.9	
IMMY LFA vs IMMY EIA	99.7	0.98 (0.96–1.00)
Positive agreement	97.4	
Negative agreement	99.9	

mens were positive and 921 (527 serum and 394 CSF) specimens were negative by all three methods. Qualitative agreement across assays was 97.7% (kappa value = 0.82; 95% confidence interval [95% CI] = 0.75 to 0.89). Concordance was highest for CSF compared to serum specimens (99.5% versus 96.4% agreement, respectively [ $P = 0.0013$ ]). Similarly, agreement was significantly greater for CrAg-negative than CrAg-positive specimens (97.9% versus 73.7% agreement, respectively [ $P < 0.0001$ ]). Endpoint titers showed little to no correlation between assays, and on average, the IMMY tests produced severalfold higher titers than the Meridian test (data not shown).

**Discrepant specimens.** Twenty-three specimens (2.3%) yielded discordant results across the assay comparisons, and all of these were Meridian EIA negative (Table 3). Of the 23 discrepant specimens, 20 (87.0%) were positive by both the IMMY LFA and EIA, 2 (8.7%) were LFA positive only, and 1 (4.3%) was EIA pos-

itive only. Eleven samples had an adequate volume for LA testing; 8/11 samples (72.7%) were LA positive, and 3/11 samples (27.3%) were LA negative. LA-negative samples (2 CSF samples and 1 serum) displayed low titers by IMMY EIA/LFA ( $\leq 1:10$ ).

The same 11 specimens analyzed by LA assay were retested using a panel of anti-GXM MAbs with distinct recognition patterns for the four major GXM serotypes (Table 3). Low-titer specimens could not be evaluated using the GXM EIA (6/11 samples [54.5%]). The pattern of MAb recognition for 4 of 5 higher-titer specimens (i.e., discrepant patient samples 785, 287, 497, and 603) was consistent with the expression of serotype C epitopes (Fig. 1B). Specimens containing serotype C-like GXM were sent to ARUP Laboratories from referring hospitals in Louisiana, California, Washington, and Missouri.

## DISCUSSION

Immunoassays designed to detect *Cryptococcus* capsular antigens are important tools for the diagnosis of cryptococcosis. We compared three commercially available CrAg assays by using a large number of serum and CSF samples that were collected as a part of routine patient care. The new IMMY LFA and EIA showed almost perfect overall agreement (kappa statistic of 0.82) with our current test, the Meridian Premier EIA. Qualitative agreement was significantly greater for CrAg-negative than CrAg-positive specimens, likely due to sensitivity differences between the IMMY and Meridian assays. In addition, interassay agreement was higher for CSF than for serum specimens. This may have been a result of higher antigen concentrations present in the CSF of patients with cryptococcal meningitis. The highest percent agreement (99.7%) was observed between the two IMMY tests, which reflects the use of the same MAbs for CrAg detection in both platforms.

TABLE 3 Discrepant specimen results<sup>a</sup>

Specimen ID no.	Source	IMMY LFA result	LFA titer	IMMY EIA result	EIA titer	Meridian EIA result	IMMY latex test result	Latex test titer	GXM serotype
058	Serum	POS	<1:5	POS	1:5	NEG	QNS	NA	NA
804	Serum	POS	1:44	POS	1:40	NEG	QNS	NA	NA
122	Serum	POS	1:35	POS	1:40	NEG	QNS	NA	NA
104	Serum	POS	1:14	POS	1:5	NEG	QNS	NA	NA
953	Serum	POS	1:17	POS	1:5	NEG	QNS	NA	NA
207	Serum	POS	1:59	POS	1:80	NEG	QNS	NA	NA
749	Serum	POS	<1:5	POS	1:5	NEG	QNS	NA	NA
894	Serum	POS	<1:5	POS	1:5	NEG	QNS	NA	NA
046	Serum	POS	<1:5	POS	1:5	NEG	QNS	NA	NA
520	Serum	POS	1:14	POS	1:5	NEG	QNS	NA	NA
806	Serum	POS	QNS	POS	1:20	NEG	QNS	NA	NA
309	Serum	NEG	NA	POS	1:5	NEG	QNS	NA	NA
777	CSF	POS	QNS	NEG	NA	NEG	NEG	NA	NA
287	Serum	POS	1:12	POS	1:5	NEG	POS	QNS	C-like
028	Serum	POS	<1:5	POS	1:5	NEG	POS	1:2	INDETERM
603	Serum	POS	1:86	POS	1:40	INDETERM	POS	1:32	C-like
963	CSF	POS	1:10	POS	1:5	NEG	NEG	NA	INDETERM
050	Serum	POS	1:27	POS	1:10	INDETERM	POS	1:8	INCON <sup>b</sup>
497	Serum	POS	1:105	POS	1:320	NEG	POS	1:32	C-like
378	Serum	POS	<1:5	POS	1:5	NEG	POS	<1:2	INDETERM
161	Serum	NEG	NA	POS	1:5	NEG	NEG	NA	INDETERM
780	Serum	POS	<1:5	POS	1:10	NEG	POS	<1:2	INDETERM
785	Serum	POS	1:62	POS	1:320	NEG	POS	1:128	C-like

<sup>a</sup> Abbreviations: POS, positive; NEG, negative; NA, not applicable; QNS, quantity of the specimen was not sufficient for repeat testing; INDETERM, indeterminate; INCON, inconclusive; GXM, glucuronoxylomannan.

<sup>b</sup> MAb reactivity was not typical of patterns observed with GXM from the four major serotypes.

The IMMY assays had the highest sensitivities, with positivity rates of 7.8% and 7.6% for the IMMY LFA and IMMY EIA, respectively, compared to the Meridian EIA positivity rate of 5.6%. Analysis of discrepant specimens by use of a separate latex agglutination kit suggested that the majority of IMMY-positive but Meridian-negative specimens with adequate volume for retesting did contain CrAg. The IMMY LFA- and/or EIA-positive specimens displayed low endpoint titers. The sensitivity of the LA method may have been influenced by the sample volume used to accommodate retesting of low-volume specimens (150  $\mu$ l versus the 300  $\mu$ l recommended in the manufacturer's instructions). An alternative explanation is that the low-titer, IMMY LFA- and/or EIA-positive results represent false-positive test results. Incomplete access to patient-level data limited our ability to fully resolve discrepancies. However, in a recent study comparing the IMMY LFA to the Meridian cryptococcal antigen latex system (CALAS), 2 of 76 specimens (2.6%) produced discordant results; both of these were IMMY-positive but Meridian-negative samples that came from patients with a previous documented history of cryptococcosis (16).

Discrepant specimen analysis using anti-GXM MAbs with differing serotype reactivity patterns was limited by the small number of specimens with adequate volume for retesting and by an overrepresentation of low-titer samples. However, most discordant specimens with high enough titers for GXM EIA analysis (4/5 samples [80%]) were shown to contain CrAg epitopes consistent with serotype C. This observation is in line with previous reports that the Meridian assays (both the EIA and CALAS kits) have reduced sensitivity for detection of purified GXM of serotype C (8). Furthermore, the IMMY assays were constructed using a combination of MAbs that recognize all 4 major serotypes (3, 8, 11).

Serotype C (i.e., *C. gattii* molecular type VGIV) has emerged as a relatively common cause of cryptococcosis in HIV-positive patients from sub-Saharan Africa (17, 18) and has also been reported in India and South America (19–21). Patient race, HIV status, and travel histories were not available to determine whether the specimens with serotype C-like reactivity patterns in this study came from patients who had either lived in or traveled to geographic regions known to harbor serotype C *Cryptococcus*. ARUP Laboratories is a large national reference laboratory, and it is conceivable that serotype C patients were originally from one of these areas. Alternatively, these results may be the result of a heretofore unrecognized complexity in expression of CrAg epitopes.

In conclusion, the IMMY assays showed excellent overall concordance with the Meridian EIA. Assay performance differences appear to be related to issues of analytic sensitivity and serotype bias. Incomplete access to patient-level data, as well as low specimen volumes, which precluded our ability to perform repeat testing, affected our ability to fully resolve discrepant results.

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## REFERENCES

- Bloomfield N, Gordon MA, Elmendorf DF, Jr. 1963. Detection of *Cryptococcus neoformans* antigen in body fluids by latex particle agglutination. *Proc. Soc. Exp. Biol. Med.* 114:64–67.

- Babady NE, Bestrom JE, Jespersen DJ, Jones MF, Beito EM, Binnicker MJ, Wengenack NL. 2009. Evaluation of three commercial latex agglutination kits and a commercial enzyme immunoassay for the detection of cryptococcal antigen. *Med. Mycol.* 47:336–338.
- 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.
- Tanner DC, Weinstein MP, Fedorciw B, Joho KL, Thorpe JJ, Reller L. 1994. Comparison of commercial kits for detection of cryptococcal antigen. *J. Clin. Microbiol.* 32:1680–1684.
- Ikeda R, Nishikawa A, Shinoda T, Fukazawa Y. 1985. Chemical characterization of capsular polysaccharide from *Cryptococcus neoformans* serotype A-D. *Microbiol. Immunol.* 29:981–991.
- Wilson DE, Bennett JE, Bailey JW. 1968. Serologic grouping of *Cryptococcus neoformans*. *Proc. Soc. Exp. Biol. Med.* 127:820–823.
- Byrnes EJ, 3rd, Bartlett KH, Perfect JR, Heitman J. 2011. *Cryptococcus gattii*: an emerging fungal pathogen infecting humans and animals. *Microbes Infect.* 13:895–907.
- Percival A, Thorkildson P, Kozel TR. 2011. Monoclonal antibodies specific for immunorecessive epitopes of glucuronoxylomannan, the major capsular polysaccharide of *Cryptococcus neoformans*, reduce serotype bias in an immunoassay for cryptococcal antigen. *Clin. Vaccine Immunol.* 18:1292–1296.
- Lindsley MD, Mekha N, Baggett HC, Surinthong Y, Autthathainchai 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.
- Belay T, Cherniak R, Kozel TR, Casadevall A. 1997. Reactivity patterns and epitope specificities of anti-*Cryptococcus neoformans* monoclonal antibodies by enzyme-linked immunosorbent assay and dot enzyme assay. *Infect. Immun.* 65:718–728.
- Brandt S, Thorkildson P, Kozel TR. 2003. Monoclonal antibodies reactive with immunorecessive epitopes of glucuronoxylomannan, the major capsular polysaccharide of *Cryptococcus neoformans*. *Clin. Diagn. Lab. Immunol.* 10:903–909.
- Eckert TF, Kozel TR. 1987. Production and characterization of monoclonal antibodies specific for *Cryptococcus neoformans* capsular polysaccharide. *Infect. Immun.* 55:1895–1899.
- Gates-Hollingsworth MA, Kozel TR. 2009. Phenotypic heterogeneity in expression of epitopes in the *Cryptococcus neoformans* capsule. *Mol. Microbiol.* 74:126–138.
- Netski D, Kozel TR. 2002. Fc-dependent and Fc-independent opsonization of *Cryptococcus neoformans* by anticapsular monoclonal antibodies: importance of epitope specificity. *Infect. Immun.* 70:2812–2819.
- Spiropulu C, Eppard RA, Ottosen E, Kozel TR. 1989. Antigenic variation within serotypes of *Cryptococcus neoformans* detected by monoclonal antibodies specific for the capsular polysaccharide. *Infect. Immun.* 57:3240–3242.
- Clarke S, Gibb R. 2012. Evaluation of the IMMY CrAg lateral flow assay for detection of cryptococcal antigen [abstract]. *Aust. Soc. Microbiol.* 2012 Annu. Sci. Meet., Brisbane, Australia.
- 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.
- Karstaedt AS, Crewe-Brown HH, Dromer F. 2002. Cryptococcal meningitis caused by *Cryptococcus neoformans* var. *gattii*, serotype C, in AIDS patients in Soweto, South Africa. *Med. Mycol.* 40:7–11.
- Cogliati M, Chandrashekar N, Esposto MC, Chandramuki A, Petrini B, Viviani MA. 2012. *Cryptococcus gattii* serotype-C strains isolated in Bangalore, Karnataka, India. *Mycoses* 55:262–268.
- Perez C, Dolande M, Moya M, Rosello A, de Capriles CR, Landaeta ME, Mata-Essayag S. 2008. *Cryptococcus neoformans*, *Cryptococcus gattii*: serotypes in Venezuela. *Mycopathologia* 166:149–153.
- Trilles L, Lazera Mdos S, Wanke B, Oliveira RV, Barbosa GG, Nishikawa MM, Morales BP, Meyer W. 2008. Regional pattern of the molecular types of *Cryptococcus neoformans* and *Cryptococcus gattii* in Brazil. *Mem. Inst. Oswaldo Cruz* 103:455–462.