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 for both the IMMY LFA and EIA, 2 were LFA positive only, and 1 was EIA positive only. Eleven discrepant samples 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 (<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.

**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 (≤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 test results and endpoint titers (<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 test results and endpoint titers (<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 test results and endpoint titers (<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 test results and endpoint titers (<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 test results and endpoint titers (<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).
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 (OD450/630) of >0.265, and a negative result was defined as an OD450/630 of ≤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, OD450/630 <0.070; indeterminate result, OD450/630 of ≥0.070 to <0.100; and positive result, OD450/630 of ≥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 μl to 150 μl, 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 EIA results 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-
mMens 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 positive 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 (<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.

**DISCUSSION**

**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        |                      |

**TABLE 3 Discrepant specimen results**

| 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           |
| 084             | 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                 | QNS                   | C-like           |              |
| 028             | Serum  | POS             | <1:5      | POS             | 1:5       | NEG                 | POS                   | QNS              |              |
| 603             | Serum  | POS             | 1:86      | POS             | 1:40      | INDETERM            | POS                   | 1:32             | C-like       |
| 963             | CSF    | POS             | 1:10      | POS             | 1:5       | NEG                 | NEG                   | INDETERM         |              |
| 050             | Serum  | POS             | 1:27      | POS             | 1:10      | INDETERM            | POS                   | 1:8              | INCON        |
| 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                   | INDETERM         |              |
| 780             | Serum  | POS             | <1:5      | POS             | 1:10      | NEG                 | POS                   | <1:2             | INDETERM     |
| 785             | Serum  | POS             | 1:62      | POS             | 1:520     | NEG                 | POS                   | 1:128            | C-like       |

**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.

**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 μl versus the 300 μl 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 C. neoformans. 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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J.H. performed and interpreted all of the LFA results for this study.

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