*Open Forum Infectious Diseases*

MAJOR ARTICLE

# Testing for Cryptococcosis at a Major Commercial Laboratory—United States, 2019–2021

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***Background.*** Cryptococcosis is a serious opportunistic fungal disease, and the proportion of cases among patients with immunosuppressive conditions other than HIV or organ transplant has increased. Understanding laboratory testing patterns for cryptococcosis is useful for estimating its true burden and developing testing guidance.

***Methods.*** We identified cryptococcosis tests (cryptococcal antigen [CrAg], cryptococcal antibody, and fungal cultures) performed at a major national commercial laboratory ordered during March 1, 2019–October 1, 2021, and analyzed test results, patient and provider features, reasons for testing, geography, and temporal trends.

***Results.*** Among 29 180 serum CrAg tests, 4422 (15.2%) were positive, and among 10 724 cerebrospinal fluid (CSF) CrAg tests, 492 (4.6%) were positive. Frequent reasons for serum CrAg testing in nonhospital settings (10 882 tests) were HIV (44.6%) and cryptococcosis (17.0%); other underlying conditions were uncommonly listed (<10% total). Serum CrAg positivity declined from 25.6% in October 2019 to 11.3% in September 2021. The South had the highest positivity for serum CrAg tests (16.6%), CSF CrAg tests (4.7%), and fungal cultures (0.15%). Among 5009 cryptococcal antibody tests, 5 (0.1%) were positive.

***Conclusions.*** Few outpatient serum CrAg tests were performed for patients with immunocompromising conditions other than HIV, suggesting potential missed opportunities for early detection. Given the high positive predictive value of CrAg testing, research is needed to improve early diagnosis, particularly in patients without HIV. Conversely, the low yield of antibody testing suggests that it may be of low value. The decline in CrAg positivity during the COVID-19 pandemic warrants further investigation.

**Keywords.** cryptococcosis; *Cryptococcus*; antigens; fungal; laboratories; United States.

Cryptococcosis is a serious invasive fungal disease associated with substantial morbidity and mortality. An estimated quarter of a million cases of cryptococcal meningitis occur yearly among people with HIV worldwide [1]. The *Cryptococcus neoformans* and *C. gattii* species complexes are the most clinically relevant causes of disease; the *C. neoformans* species complex is classically associated with central nervous system infections, and *C. gattii* species complex more often causes pulmonary infections, although both species can cause various clinical manifestations [2]. Cryptococcosis primarily affects persons with immunosuppression, particularly advanced HIV [3]. In the United States, the proportion of cryptococcosis cases in people with HIV has declined in recent years, whereas the proportion of cases in solid organ transplant recipients and the non-HIV, nontransplant population has increased, accounting for over a third of cryptococcosis patients; this epidemiologic shift has been relatively well characterized, mainly using administrative data or single-center cohort studies [4–7]. Cryptococcosis in apparently immunocompetent patients has also been increasingly recognized [6,8,9]. Public health surveillance is limited to only 3 states (Louisiana, Oregon, and Washington). Therefore, the total burden of cryptococcosis in the United States remains largely undefined and is likely larger than the ~5000 cryptococcosis-associated hospitalizations documented each year through administrative coding [10].

Because early detection and treatment of cryptococcosis can reduce morbidity and mortality [11], understanding testing patterns for cryptococcosis is an essential part of estimating its public health burden. Laboratory methods to diagnose cryptococcosis typically include culture; microscopic examination of cerebrospinal fluid (CSF) or tissue; and cryptococcal antigen (CrAg) detection in body fluids by latex agglutination, enzyme immunoassay, or lateral flow assay (LFA). The semiquantitative CrAg LFA is a simple, rapid, highly accurate, and inexpensive diagnostic method. It can be also used to detect early, asymptomatic cryptococcal infection; in the United States, screening is recommended for persons with newly diagnosed HIV and CD4 counts ≤100 cells/mm$^3$ [12]. Molecular diagnosis of cryptococcal meningitis is also

Received 15 March 2022; editorial decision 3 May 2022; accepted 12 May 2022; published online 16 May 2022

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https://doi.org/10.1093/ofid/ofac253

possible with the BioFire FilmArray Meningitis/Encephalitis Panel [13]. Cryptococcal antibody testing exists but cannot diagnose cryptococcosis given its limited accuracy and potential for cross-reactions with other fungi [14,15].

Despite the recent epidemiologic changes in patient populations most often affected by cryptococcosis and the variety of available diagnostic methods, nationwide testing patterns have not been well described, apart from a decade-old survey of infectious disease physicians' self-reported practices [16]. We analyzed a national commercial laboratory data set to better characterize testing patterns for cryptococcosis, yielding greater insight into the burden of this disease and identifying opportunities to improve testing practices.

## METHODS

### Data Source

The Centers for Disease Control and Prevention's (CDC's) National Syndromic Surveillance Program (NSSP) collects data from a major national commercial laboratory ("Laboratory A") on testing performed for all reportable diseases in the United States. If a condition is reportable in any US jurisdiction, Laboratory A transmits nationwide test orders and results for that condition to the NSSP; Laboratory A transmits these data to the NSSP every 10 minutes via HL7 message. Data include all result types (eg, positive, negative, test not performed). Limited patient demographic data are included; however, no unique patient identifier is available, and distinguishing repeat tests for the same patient is not possible.

### Analysis

We used Logical Observation Identifiers Names and Codes (LOINC) codes to identify CrAg testing (70910-5, 70911-3), cryptococcal antibody testing (6369-3), and fungal cultures (601-5, 580-1, 569-4, 568-6, 51723-5, 42804-5, 18482-0, 17949-9, 17948-1, 17947-3) ordered during March 1, 2019 (earliest available data)–October 1, 2021. For all cryptococcosis-specific tests and fungal cultures positive for *Cryptococcus*, we analyzed patient demographic features, US Census region and specialty of the ordering provider, and temporal trends in number of tests and percent positivity. For cryptococcal antigen and antibody tests, we also examined reason for testing (RFT), which is captured by up to 6 International Classification of Diseases, 10th Revision, Clinical Modification (ICD-10-CM) codes. We selected specific ICD-10-CM codes for underlying conditions that can increase risk for developing cryptococcosis, as well as codes for symptoms and clinical findings associated with cryptococcosis (Supplementary Table 1). For *Cryptococcus* cultures, we examined species-specific patient demographic features, US census region and specialty of the ordering provider, and specimen type.

## RESULTS

### Serum and CSF CrAg Testing

Total, 29 180 serum CrAg tests were ordered, of which 4422 (15.2%) were positive, and 10 724 CSF CrAg tests were ordered, of which 492 (4.6%) were positive (Table 1). Among all CrAg tests, the median patient age (interquartile range [IQR]) was 55 (40–67) years, and 58.8% of tests were among males; 73.9% of the positive tests were among males. Half (50.0%) of serum CrAg tests were ordered from a hospital setting, 24.8% were ordered by outpatient infectious disease providers, and 10% by family/general practitioners or internal medicine providers.

RFT was recorded for 49.8% of all serum CrAg tests and 12.6% of CSF CrAg tests; 4.8% of tests ordered from hospitals had RFT recorded vs 90.2% of tests ordered from nonhospital settings. Among nonhospital serum CrAg tests, the most frequent underlying conditions we identified as RFT were HIV (44.6%) and cryptococcosis (17.0%); transplant (1.7%) and other specific underlying conditions were infrequently listed. Respiratory symptoms (17.3%) were more commonly listed as serum CrAg RFT in nonhospital settings than systemic symptoms (6.0%) or those related to the central nervous system (2.7%).

The number of serum CrAg tests ordered remained relatively constant over time; however, the percent positivity declined from a peak of 25.6% in October 2019 to 11.3% in September 2021 (Figure 1). Similar trends in percent positivity occurred among different RFT groups (with or without HIV, with or without cryptococcosis, and without HIV or cryptococcosis) (Supplementary Figure 1). The overall percent positivity among serum CrAg tests with cryptococcosis listed as RFT was 72.1%, with HIV was 22.5%, and without HIV or cryptococcosis was 6.1%.

Percent positivity was highest in the South for both serum (16.6%) and CSF CrAg tests (4.7%) (Table 2). Among serum CrAg tests with HIV listed as an RFT, the highest proportion of tests was ordered in the South (67.3%), but the highest positivity rate occurred in the Midwest (38.2%).

### Fungal Cultures

Among 804 086 fungal cultures, 69.9% were negative, 27.1% were positive for another organism besides *Cryptococcus*, and 1014 (0.1%) were positive for *Cryptococcus*. *C. neoformans* was the most common species (77.3%), followed by *C. albidus* (6.6%), *C. laurentii* (4.9%), and *C. gattii* (3.6%) (Table 3). Among all cultures positive for *Cryptococcus*, the median patient age (IQR) was 53 (38–68) years. Most (68.6%) cultures were from males; however, most (56.7%) *C. albidus* cultures were from females. Most (80.5%) *Cryptococcus* cultures were ordered from a hospital setting, although dermatology was a frequent ordering specialty for *C. albidus*.

Specimen type was missing, unknown, or an isolate from an unknown body site in 48.9% of *Cryptococcus* cultures. CSF was

**Table 1. Cryptococcal Antigen Tests Performed by Laboratory A, March 1, 2019–October 1, 2021**

| | Serum | | | Cerebrospinal Fluid | | |
|---|---|---|---|---|---|---|
| | No. Tested | Positive Tests, No. (%) | Negative Tests, No. (%) | No. Tested | Positive Tests, No. (%) | Negative Tests, No. (%) |
| Total | 28 809 | 4422 (15.2) | 24 387 (83.6) | 10 462 | 492 (4.6) | 9970 (93.1) |
| Median age (IQR), y | 28 760 | 54.0 (42–64) | 55.5 (40–67) | 10 356 | 49.0 (36–58) | 53.0 (38–67) |
| Sex | | | | | | |
| Male | 18 033 | 3227 (73.1) | 14 806 (60.8) | 4964 | 396 (81.1) | 4568 (46.3) |
| Female | 10 723 | 1185 (26.9) | 9538 (39.2) | 5383 | 92 (18.9) | 5291 (53.7) |
| Ordering specialty | 23 568 | 3591 (81.2) | 19 977 (81.9) | 8360 | 380 (77.2) | 7980 (80.0) |
| Family/general practice | 1159 | 193 (5.4) | 966 (4.8) | 24 | 2 (0.5) | 22 (0.3) |
| Hospital | 11 776 | 1233 (34.3) | 10 543 (52.8) | 7128 | 356 (93.7) | 6772 (84.9) |
| Infectious disease | 5855 | 1422 (39.6) | 4433 (22.2) | 4 | 0 (0.0) | 4 (0.1) |
| Internal medicine | 1173 | 254 (7.1) | 919 (4.6) | 21 | 0 (0.0) | 21 (0.3) |
| Other | 3605 | 489 (13.6) | 3116 (15.6) | 1183 | 22 (5.8) | 1161 (14.5) |
| Reason for testing[a] | 10 882 | 2155 (48.7) | 8727 (35.8) | | | |
| Underlying conditions | | | | | | |
| Cryptococcosis | 1847 | 1332 (61.8) | 515 (5.9) | | | |
| HIV | 4853 | 1092 (50.7) | 3761 (43.1) | | | |
| Asymptomatic HIV | 321 | 20 (0.9) | 301 (3.4) | | | |
| Transplant | 186 | 63 (2.9) | 123 (1.4) | | | |
| Immune-mediated inflammatory disease | 113 | 10 (0.5) | 103 (1.2) | | | |
| COVID-19 | 24 | 3 (0.1) | 21 (0.2) | | | |
| Solid cancer | 208 | 51 (2.4) | 157 (1.8) | | | |
| Hematologic malignancy | 56 | 19 (0.9) | 37 (0.4) | | | |
| Chronic kidney disease or kidney failure | 128 | 55 (2.6) | 73 (0.8) | | | |
| Liver disease or liver failure | 96 | 11 (0.5) | 85 (1.0) | | | |
| Diabetes | 199 | 59 (2.7) | 140 (1.6) | | | |
| Symptoms and clinical findings | | | | | | |
| Systemic | 653 | 42 (1.9) | 611 (7.0) | | | |
| Respiratory | 1879 | 139 (6.5) | 1740 (19.9) | | | |
| Central nervous system | 293 | 39 (1.8) | 254 (2.9) | | | |
| Titer[b] | | | | | | |
| <1:5 (negative) | | 1 (0.0) | | | 0 (0.0) | |
| 1:5 | | 436 (9.9) | | | 19 (3.9) | |
| 1:10 | | 407 (9.2) | | | 14 (2.9) | |
| 1:20 | | 382 (8.6) | | | 19 (3.9) | |
| 1:40 | | 378 (8.5) | | | 23 (4.7) | |
| 1:80 | | 356 (8.1) | | | 15 (3.1) | |
| 1:160 | | 279 (6.3) | | | 25 (5.1) | |
| 1:320 | | 246 (5.6) | | | 30 (6.1) | |
| 1:512 | | 0 (0.0) | | | 7 (1.4) | |
| 1:640 | | 206 (4.7) | | | 28 (5.7) | |
| 1:1280 | | 186 (4.2) | | | 32 (6.5) | |
| 1:2560 | | 542 (12.3) | | | 94 (19.1) | |
| 1:5120 | | 3 (0.1) | | | 0 (0.0) | |
| >1:5120 | | 536 (12.1) | | | 98 (20.0) | |
| Missing/unknown | | 464 (10.5) | | | 87 (17.7) | |

Abbreviations: COVID-19, coronavirus disease 2019; CrAg, cryptococcal antigen; IQR, interquartile range; RFT, reason for testing.

[a]Among tests ordered from settings other than hospitals. RFT classifications were not mutually exclusive.

[b]Within Laboratory A, positive CrAg results trigger reflex testing for CrAg titers.

the most commonly specified specimen type, in 30.6% of *C. neoformans* cultures and 50.0% of *C. gattii* cultures; 88.2% of *Cryptococcus* cultures from CSF were *C. neoformans*. *C. laurentii* cultures were also frequently from potentially invasive (blood, CSF, respiratory fluid, or lung tissue) body sites (30.0%), whereas skin was the most commonly specified specimen type for *C. albidus* cultures (29.9%). No *C. albidus* cultures were from CSF.

By month, the number of *Cryptococcus* cultures varied from 12 in May 2020 to 63 in December 2020, and the percentage of

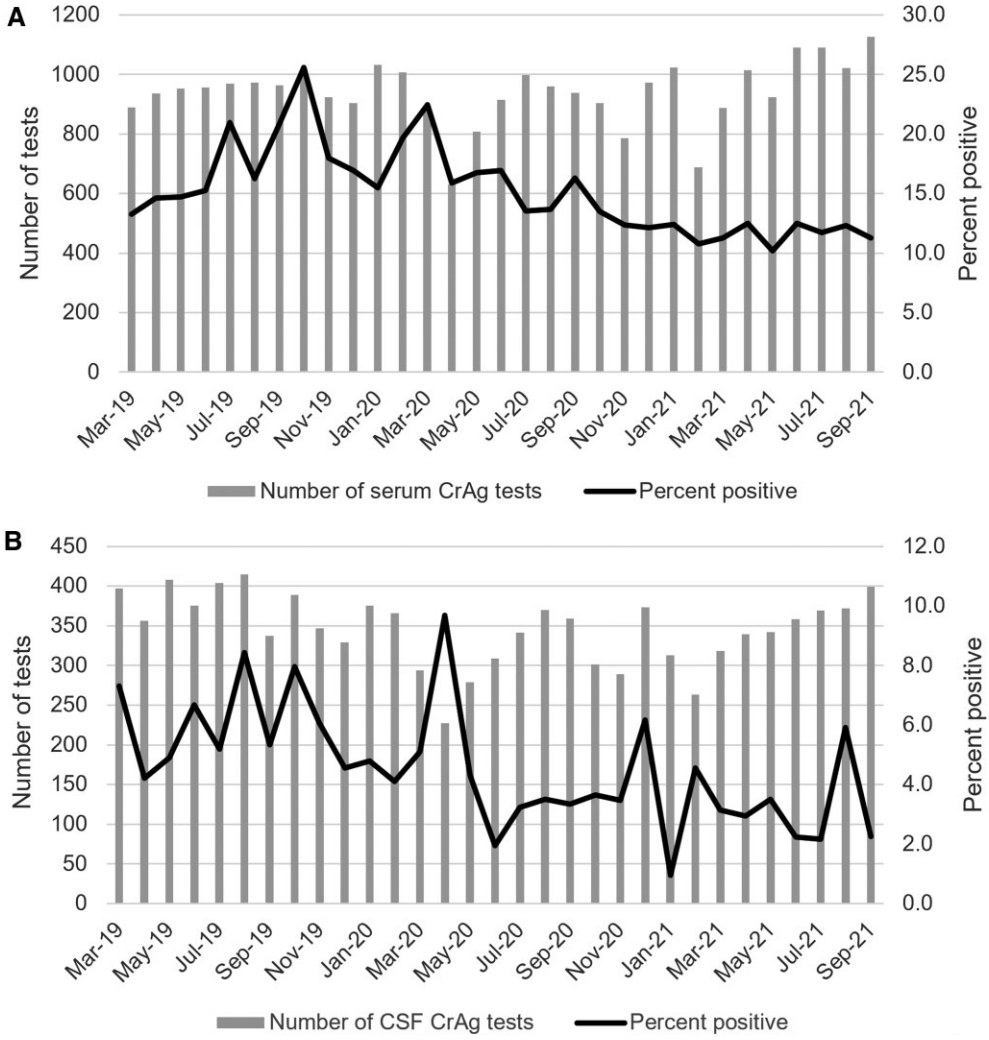


**Figure 1.** Monthly number and percent positive (A) serum and (B) CSF CrAg tests performed by Laboratory A, March 1, 2019–October 1, 2021. Abbreviations: CrAg, cryptococcal antigen; CSF, cerebrospinal fluid.

all fungal cultures positive for *Cryptococcus* ranged from 0.06% in May 2020 to 0.27% in April 2020 (Supplementary Figure 2). More than half (52.5%) of all fungal cultures and 62.8% of *Cryptococcus* cultures were ordered by providers in the South, and the South had the highest proportion of fungal cultures positive for *Cryptococcus* (0.15%) (Table 2). By species, most *C. neoformans* (70.4%), *C. gattii* (64.9%), and *C. laurentii* (60.0%) cultures were from the South, whereas most *C. albidus* cultures (77.6%) were from the Northeast.

### Cryptococcal Antibody Testing

Among 5009 cryptococcal antibody tests ordered, 5 (0.1%) were positive (Table 4). Cryptococcal antibody tests were most commonly ordered from a hospital setting (58.3%) and in the South (53.4%). Among the 39.3% of cryptococcal antibody tests with RFT recorded, an abnormal finding on imaging was the most common RFT (21.2%), followed by HIV (15.8%). By month, the number of cryptococcal antibody tests ranged from 95 in April 2020 to 211 in June 2021 (Supplementary Figure 3).

## DISCUSSION

This analysis of data from a large commercial laboratory system provides preliminary insight into national testing patterns for cryptococcosis. ICD-10-CM diagnosis codes listed as reasons for CrAg testing in nonhospital settings support the known association between HIV and cryptococcal infection, but other patient populations did not appear to be as well represented, contrasting with the known burden of cryptococcosis in people without HIV. These findings suggest that more work is needed to identify optimal outpatient testing strategies to identify cryptococcosis before severe disease occurs in non-HIV patients. We observed an overall decline over time in CrAg positivity

**Table 2. Cryptococcal Antigen Tests and *Cryptococcus* Cultures by Region at Laboratory A, March 1, 2019–October 1, 2021**

| | Total | Midwest | Northeast | South | West | Unknown |
|---|---|---|---|---|---|---|
| Serum CrAg tests | | | | | | |
| No. (%) positive | 4422 (15.2) | 258 (16.3) | 442 (14.7) | 2499 (16.6) | 1167 (12.7) | 56 (16.1) |
| Test ordering rate per 100 000 population[a] | 8.8 | 2.3 | 5.2 | 11.9 | 11.7 | n/a |
| Proportion of tests ordered by region, % | | 5.4 | 10.3 | 51.5 | 1.2 | 31.6 |
| CSF CrAg tests | | | | | | |
| No. (%) positive | 492 (4.6) | 23 (2.3) | 39 (4.3) | 277 (4.7) | 122 (4.6) | 31 (12.3) |
| Test ordering rate per 100 000 population[a] | 3.2 | 1.4 | 1.6 | 4.7 | 3.3 | n/a |
| Proportion of tests ordered by region, % | | 9.2 | 8.4 | 55.5 | 24.5 | 2.4 |
| Serum CrAg tests with HIV listed as a reason for testing | | | | | | |
| No. positive (% positive) | 1340 (21.5) | 73 (38.2) | 104 (19.9) | 769 (18.3) | 394 (29.6) | 0 (0.0) |
| Test ordering rate per 100 000 population[a] | 1.9 | 0.3 | 0.9 | 3.3 | 1.7 | n/a |
| Proportion of tests ordered by region, % | | 3.1 | 8.4 | 67.3 | 21.3 | 0.0 |
| Serum CrAg tests without HIV listed as a reason for testing | | | | | | |
| No. (%) positive | 1372 (16.6) | 70 (11.7) | 122 (18.7) | 832 (17.9) | 348 (14.6) | 0 (0.0) |
| Test ordering rate per 100 000 population[a] | 2.5 | 0.9 | 1.1 | 3.7 | 3.0 | n/a |
| Proportion of tests ordered by region, % | | 7.2 | 7.9 | 56.0 | 28.8 | 0.0 |
| *Cryptococcus* cultures | | | | | | |
| No. (%) positive out of all fungal cultures ordered | 1014 (0.13) | 71 (0.10) | 150 (0.10) | 637 (0.15) | 146 (0.09) | 10 (0.13) |
| Fungal culture ordering rate per 100 000 population[a] | 242.6 | 102.7 | 251.5 | 334.3 | 202.5 | n/a |
| Proportion of fungal cultures ordered by region, % | | 8.8 | 18.0 | 52.5 | 19.7 | 1.0 |

Abbreviations: CrAg, cryptococcal antigen; CSF, cerebrospinal fluid.

[a]Test ordering rates per 100 000 population were calculated using 2020 Decennial Census data, available at: https://data.census.gov/cedsci/all?q=2020&d=DEC%20Redistricting%20Data%20%28PL%2094-171%29.

**Table 3. Fungal Cultures Positive for *Cryptococcus* Performed by Laboratory A, March 1, 2019–October 1, 2021**

| | Total, No. (%) | *C. neoformans*, No. (%) | *C. albidus*, No. (%) | *C. laurentii*, No. (%) | *C. gattii*, No. (%) | Other Specified *Cryptococcus* Species, No. (%) | Unspecified *Cryptococcus* Species, No. (%) |
|---|---|---|---|---|---|---|---|
| Total | 1014 (100.0) | 784 (77.3) | 67 (6.6) | 50 (4.9) | 36 (3.6) | 46 (4.5) | 31 (3.1) |
| Median age (IQR), y (n = 1002) | 53 (38–68) | 53 (39-68) | 57 (32–76) | 50 (32–63) | 41 (35–46) | 46 (28–66) | 66 (55–74) |
| Sex | 996 | 767 | 67 | 49 | 36 | 46 | 31 |
| Male | 683 (68.6) | 557 (72.6) | 29 (43.3) | 23 (46.9) | 31 (86.1) | 25 (54.3) | 18 (58.1) |
| Female | 313 (31.4) | 210 (27.4) | 38 (56.7) | 26 (53.1) | 5 (13.9) | 21 (45.7) | 13 (41.9) |
| Ordering specialty | 843 | 661 | 56 | 40 | 26 | 38 | 22 |
| Hospital | 679 (80.5) | 599 (90.6) | 9 (16.1) | 19 (47.5) | 25 (96.2) | 14 (36.8) | 13 (59.1) |
| Dermatology | 63 (7.5) | 4 (0.6) | 35 (62.5) | 8 (20.0) | 0 (0.0) | 14 (36.8) | 2 (9.1) |
| Other | 101 (12.0) | 58 (8.8) | 12 (21.4) | 13 (32.5) | 1 (3.8) | 10 (26.3) | 7 (31.8) |
| Specimen type | | | | | | | |
| Blood | 87 (8.6) | 79 (10.1) | 0 (0.0) | 6 (12.0) | 0 (0.0) | 1 (2.2) | 1 (3.2) |
| CSF | 272 (26.8) | 240 (30.6) | 0 (0.0) | 7 (14.0) | 18 (50.0) | 7 (15.2) | 0 (0.0) |
| Isolate from unknown body site | 182 (17.9) | 159 (20.3) | 1 (1.5) | 7 (14.0) | 8 (22.2) | 2 (4.3) | 5 (16.1) |
| Nail | 5 (0.5) | 0 (0.0) | 1 (1.5) | 1 (2.0) | 0 (0.0) | 3 (6.5) | 0 (0.0) |
| Other | 24 (2.4) | 16 (2.0) | 2 (3.0) | 0 (0.0) | 4 (11.1) | 2 (4.3) | 0 (0.0) |
| Respiratory fluid or lung tissue | 88 (8.7) | 75 (9.6) | 2 (3.0) | 2 (4.0) | 1 (2.8) | 0 (0.0) | 8 (25.8) |
| Skin | 42 (4.1) | 5 (0.6) | 20 (29.9) | 4 (8.0) | 1 (2.8) | 10 (21.7) | 2 (6.5) |
| Missing or unknown | 314 (31.0) | 210 (26.8) | 41 (61.2) | 23 (46.0) | 4 (11.1) | 21 (45.7) | 15 (48.4) |
| Census region | | | | | | | |
| Midwest | 71 (7.0) | 54 (6.9) | 2 (3.0) | 4 (8.0) | 0 (0.0) | 3 (6.5) | 8 (25.8) |
| Northeast | 150 (14.8) | 52 (6.6) | 52 (77.6) | 14 (28.0) | 1 (2.8) | 28 (60.9) | 3 (9.7) |
| South | 637 (62.8) | 552 (70.4) | 6 (9.0) | 30 (60.0) | 25 (69.4) | 10 (21.7) | 14 (45.2) |
| West | 146 (14.4) | 118 (15.1) | 7 (10.4) | 2 (4.0) | 10 (27.8) | 3 (6.5) | 6 (19.4) |
| Unknown | 10 (1.0) | 8 (1.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (4.3) | 0 (0.0) |

Abbreviations: CSF, cerebrospinal fluid; IQR, interquartile range.

**Table 4. Cryptococcal Antibody Tests Performed by Laboratory A, March 1, 2019–October 1, 2021**

| | No. (%) |
|---|---|
| Total | 5009 |
| Result | |
| Negative | 4937 (98.6) |
| Positive | 5 (0.1) |
| Test not performed | 67 (1.3) |
| Median age (IQR), y (n = 4938) | 57 (42–68) |
| Sex (n = 4939) | |
| Male | 2699 (54.6) |
| Female | 2240 (45.4) |
| Ordering specialty (n = 4023) | |
| Family/general practice | 243 (6.0) |
| Hospital | 2347 (58.3) |
| Infectious disease | 296 (7.4) |
| Internal medicine | 329 (8.2) |
| Other | 808 (20.1) |
| Census region | |
| Midwest | 611 (12.2) |
| Northeast | 447 (8.9) |
| South | 2676 (53.4) |
| West | 1147 (22.9) |
| Unknown | 128 (2.6) |
| Reason for testing | 1972 (39.4) |
| Underlying conditions | |
| Cryptococcosis | 123 (6.2) |
| HIV | 312 (15.8) |
| Asymptomatic HIV | 35 (1.8) |
| Transplant | 18 (0.9) |
| Immune-mediated inflammatory disease | 43 (2.2) |
| COVID-19 | 3 (0.2) |
| Solid cancer | 45 (2.3) |
| Hematologic malignancy | 11 (0.6) |
| Chronic kidney disease or kidney failure | 78 (4.0) |
| Liver disease or liver failure | 24 (1.2) |
| Diabetes | 22 (1.1) |
| Symptoms and clinical findings | |
| Systemic | 123 (6.2) |
| Respiratory | 643 (32.6) |
| Abnormal imaging findings | 419 (21.2) |
| Central nervous system | 22 (1.1) |

Abbreviations: COVID-19, coronavirus disease 2019; IQR, interquartile range.

during the onset of the COVID-19 pandemic and a surprisingly high amount of cryptococcal antibody test ordering with almost no positives. These results indicate that improved guidance for cryptococcal testing is needed, which may help improve early detection of cryptococcosis.

Few comparison data are available regarding the prevalence of cryptococcosis and cryptococcal antigenemia in the United States. A landmark study of stored serum samples from HIV-infected persons with CD4 counts <100 cells/mm$^3$ found a CrAg prevalence of 2.9% [17]. Cryptococcal tests in this data set were probably performed for screening for asymptomatic infection, diagnosis among patients with clinical suspicion for cryptococcosis, and monitoring of documented cryptococcosis, likely explaining the higher percent positivity we observed (15.2%). The serum CrAg positivity rate in this analysis is also higher than in a study from an urban hospital in the Southeastern United States (6.4%), although the CSF CrAg positivity rate in our analysis (4.6%) was similar to that report (5.8%) [18]. In a similar study, only 1.9% of all patients with CrAg tests performed at a large health care system in Wisconsin tested positive [19]. Several factors may explain these differences, including possible regional differences in cryptococcosis prevalence, differences in patient populations, and differences in testing practices, including possible serial monitoring of patients with diagnosed cryptococcosis.

Many CrAg tests in this data set did not have any ICD-10-CM diagnosis codes recorded as reasons for ordering cryptococcal testing, with major variation by provider setting (90% of tests from nonhospital settings vs 5% of tests from hospitals contained this information), likely reflecting differences in billing or reimbursement requirements. Our finding that HIV was the most common ICD-10-CM diagnosis code listed as the reason for ordering serum CrAg testing in nonhospital settings is consistent with the continued risk of cryptococcosis for people with HIV, as well as the recommendation to perform CrAg testing on patients with CD4 counts ≤100 cells/mm$^3$. Unfortunately, we could not distinguish between serum CrAg tests conducted to screen HIV-infected persons for asymptomatic cryptococcal infection and those conducted to test patients with symptoms of cryptococcosis or for monitoring response to therapy, nor were data available about other clinical or laboratory features.

Immunosuppressive conditions besides HIV were not commonly listed as reasons for testing, with transplant listed on <3% of serum CrAg tests and all other underlying conditions (besides cryptococcosis) combined listed on <10%. These proportions may be influenced by the aforementioned variation in data availability by provider setting, but are somewhat lower than expected given that patients without HIV now comprise a substantial proportion of cryptococcosis cases; 1 study showed that nearly 44% of cryptococcosis-related hospitalizations occurred in patients without HIV during 2004–2012 [4]. Overall, cryptococcosis cases appear to have declined in the past several decades, largely driven by a decline in HIV-associated cases, yet cryptococcosis continues to pose considerable risk for many patient groups [4,5]. Our findings could indicate that non-HIV immunosuppressive conditions are simply not commonly being coded as reasons for cryptococcal testing or that providers are not testing for cryptococcosis in patients without HIV as often as for patients with HIV. In the context of serum CrAg testing for asymptomatic antigenemia, this is expected as cryptococcal screening is not routinely recommended for patients without HIV. The high percent positivity we observed among certain non-HIV groups may reflect

repeat testing, but it may also point toward missed opportunities for early diagnosis of cryptococcosis in patients without HIV, consistent with other studies that show diagnostic delays for these patients [20]. Such delays are partly responsible for increased morbidity, mortality, and medical costs compared with cryptococcosis patients with HIV [4–7,20].

Our findings indicate that studies are needed to assess strategies for outpatient detection of cryptococcosis to prevent severe disease, taking into account the high positive predictive value (PPV) and low cost of CrAg testing. For example, serum CrAg positivity among tests ordered from nonhospital settings was 6.1% among tests without HIV or cryptococcosis codes listed, suggesting that a crude number needed to test would be ∼1 in 17. The PPV of CrAg is nearly 100%, and the cost has been documented to be as low as $10 per test [11]. Even assuming a cost of $30 per test, the cost to detect 1 positive in this population would be only $510 (ie, 17 patients at $30 each). Further study is needed to determine the impact on hospitalization and death of treatment of a positive outpatient CrAg test in certain non-HIV populations, but experiences from HIV suggest that such interventions could be highly cost-effective [11].

Overall, cryptococcosis was the second most common condition listed as a reason for testing that we examined, noted for a high proportion of positive CrAg tests and a low proportion of negative CrAg tests. This likely indicates that providers or medical coders were incorrectly listing cryptococcosis diagnosis codes as a reason for testing when the patient did not yet have a cryptococcosis diagnosis, or that many CrAg tests were performed for monitoring patients with known cryptococcosis. Notably, changes in CrAg titers do not necessarily correlate with clinical response, and CrAg can remain detectable for months to years following successful antifungal treatment [21,22]. Indeed, clinical deterioration may be due to immunologic factors such as immune reconstitution syndromes in patients with HIV or postinfectious inflammatory response syndromes in patients without HIV [23]. Therefore, serial monitoring of serum CrAg during induction and consolidation treatment is generally not recommended, though it may be useful in certain situations for deciding when to discontinue or restart maintenance therapy [3,24]. The inability to identify retesting among individual patients and the lack of antifungal treatment in this data set are unfortunate barriers to understanding how repeated CrAg testing is being used to monitor patients with known cryptococcosis.

The geographic patterns we observed could be related to differences in the environmental distribution of *Cryptococcus* and the burden of cryptococcosis, underlying predisposing conditions (eg, HIV), provider testing practices, access to in-house laboratory testing for cryptococcosis, or geographic variation in use of Laboratory A. We suspect that the geographic distribution of CSF CrAg tests and *Cryptococcus* cultures may be more representative of differences in disease burden than testing practices as they are probably less likely than serum CrAg testing to represent repeat testing of the same patients over time. Although we were unable to account for the catchment distribution of the Laboratory A system, our results are consistent with the higher burden of new HIV diagnoses in the South and with the known distribution of cryptococcosis-related hospitalizations [5,25]. However, our finding of highest percent positivity in the Midwest among serum CrAg tests with HIV listed as a reason for testing was unexpected.

Nearly 20% of cultures were known to be caused by *Cryptococcus* species other than *neoformans* or *gattii*, slightly lower than the 30% of patients found in a previous retrospective analysis of *Cryptococcus* culture data from 3 Mayo Clinic locations [26]. In our study, *C. albidus* was the most common non-*neoformans*/non-*gattii* species and appeared to primarily cause noninvasive infections; alternatively, these results could suggest contamination [27]. *C. laurentii*, however, mainly affected internal body sites with a similar frequency as *C. gattii*, suggesting that *C. laurentii* may be a more common species responsible for cryptococcosis than previously recognized [26]. Unlike other studies documenting that *C. gattii* typically causes pulmonary manifestations [2,28], over half of the *C. gattii* cultures in this analysis were from CSF. Possible reasons for these unexpected findings include differences in patient populations or geography, as this analysis covers a wider geographic area than many of the previous clinically focused studies of *C. gattii* [2,28]. Broader public health surveillance or studies combining laboratory testing data and clinical data from geographically diverse areas would allow for a better understanding of the epidemiologic differences between *Cryptococcus* species.

We noted a slight decrease in the number of CrAg tests, *Cryptococcus* cultures, and cryptococcal antibody tests in Spring 2020, coinciding with stay-at-home orders to prevent transmission of COVID-19. Overall, our findings about testing frequency are generally consistent with a survey of infectious disease physicians who reported that their testing practices for pulmonary cryptococcosis did not change during the COVID-19 pandemic [29]. The steady decline in CrAg test percent positivity throughout the analytic period is somewhat puzzling. The decline may reflect fewer visits to health care providers for routine medical care or non-COVID-related illnesses [30], though the fact that the decline in positivity was greater than the decline in testing suggests that the change was not solely related to overall visits. Laboratory A experienced a decrease in routine screening for HIV and HIV-1 viral load monitoring in Spring 2020 and continued to see lower testing rates throughout 2020 compared with 2019, suggesting possible missed HIV diagnoses and reduced HIV monitoring [31]. Similarly, the COVID-19 pandemic has likely resulted in fewer HIV-infected patients undergoing screening for asymptomatic cryptococcal antigenemia or receiving follow-up testing for documented cryptococcosis, though our results did

not reveal any major decline in CrAg tests with cryptococcosis listed as a reason for testing. The direct association between COVID-19 and cryptococcosis is unclear, with few published reports of coinfections [32]; similarly, our results did not show COVID-19 commonly listed as a reason for cryptococcosis testing during the COVID-19 era. Trends in test positivity for cryptococcosis warrant further attention and investigation.

A surprisingly high number of cryptococcal antibody tests were ordered, with an extremely low positivity rate (0.1%), demonstrating that this test is unlikely to be useful for most patients. The low positivity rate is puzzling given that some background positivity among uninfected persons might be expected. One possible explanation, albeit a highly unlikely one, is that nearly all tests might have been performed for patients with immunocompromising conditions that might impair ability to mount an antibody response [14]. Our finding that "abnormal imaging result" was commonly listed as a reason for cryptococcal antibody testing may suggest that providers who order this test are attempting to determine the etiology of pulmonary nodules or other nonspecific findings on lung imaging. Our findings support the notion that cryptococcal antibody testing has limited clinical utility, suggesting that its use and availability should be carefully evaluated. Clearly noting its limitations in laboratory test ordering systems may help decrease inappropriate cryptococcal antibody testing.

Altogether, these results suggest that updates to cryptococcosis testing guidance may be warranted. First, given the extraordinarily low rate of cryptococcal antibody positivity and lack of apparent clinical utility, use of this test should be discouraged unless compelling data can be presented to support it. Second, the quantity of CrAg testing among outpatients with conditions other than HIV was far lower than for patients with HIV despite the substantial burden of cryptococcosis in patients without HIV, suggesting that clinicians should consider ordering CrAg testing more often in patients who present with respiratory or neurologic symptoms and have certain medical conditions other than HIV. Studies are needed to better determine for which of these conditions asymptomatic cryptococcal screening and treatment may be useful.

This analysis includes data from only 1 commercial laboratory system and is not a complete description of laboratory testing for cryptococcosis in the United States, nor does it necessarily represent testing patterns at other commercial laboratories, academic laboratories, or public health laboratories, all of which perform testing for cryptococcosis. Unfortunately, the proportion of nationwide cryptococcal testing performed within Laboratory A is unknown. Furthermore, the total population served by Laboratory A is also unknown and may have changed during the study period if they acquired or dropped health care systems. Another primary limitation of this analysis is the result-level, rather than patient-level, nature of the data. Lastly, we did not have access to microscopy or histopathology result data; however, these tests are likely less commonly performed for cryptococcosis than antigen testing or culture [16].

Further exploration of other laboratory data sources, in combination with more robust clinical data and reasons for testing, would be useful for a deeper understanding of testing practices and identifying potential missed opportunities for earlier cryptococcal diagnosis, particularly among patients without HIV. Perhaps our most important finding is the paucity of outpatient CrAg testing for patients without HIV who have underlying conditions increasingly implicated in severe cryptococcosis, underscoring the need to assess the costs and benefits of wider-scale testing with these highly accurate and low-cost tests.

## Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online.

## Acknowledgments

We thank Alexander Jordan for manuscript review.

***Financial support.*** This research was supported in part by the Intramural Research Program of the National Institutes of Health (AI001123 and AI001124).

***Disclaimer.*** The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

***Potential conflicts of interest.*** All authors: no reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

***Ethical approval.*** This activity was reviewed by the CDC and was conducted consistent with applicable federal law and CDC policy (45 C.F.R. part 46, 21 C.F.R. part 56; 42 U.S.C. 241(d); 5 U.S.C. 552a; 44 U.S.C. 3501 et seq).

***Patient consent.*** This study did not include factors necessitating patient consent.

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