Diagnostic accuracy of the Biosynex CryptoPS cryptococcal antigen semi-quantitative lateral flow assay in patients with advanced HIV disease

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Abstract (250/250 words)

Background: High cryptococcal antigen (CrAg) titers in blood are associated with subclinical meningitis and mortality in CrAg-positive individuals with advanced HIV-disease (AHD). We evaluated a novel semi-quantitative lateral flow assay (LFA), CryptoPS, that may be able to identify individuals with high CrAg titers in a cohort of AHD patients undergoing CrAg screening.

Methods: In a prospective cohort of patients with AHD (CD4 ≤200 cells/μL) receiving CD4 count testing, CryptoPS and IMMY LFA CrAg testing were performed on whole blood by two operators blinded to results of the other assay. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of CryptoPS were assessed against IMMY LFA as a reference. CryptoPS low-titer (T1 band) and high-titer (T2 band) results were compared against IMMY LFA titers obtained through serial dilution.

Results: 916 specimens were tested. Sensitivity of the CryptoPS assay was 61.0% (25/41, 95% confidence interval [95%CI]: 44.5-75.8), specificity 96.6% (845/875, 95%CI: 95.1-97.7), PPV 45.5% (95%CI: 32.0-59.4), and NPV 98.1% (95%CI: 97.0-98.9). All (16/16) CryptoPS false-negatives were samples with IMMY titers ≤1:160. Of 29 patients (30 specimens) who tested positive on CryptoPS but negative on IMMY LFA, none developed cryptococcal meningitis over 3-months follow-up without fluconazole. Median CrAg titers were 1:20 (interquartile range [IQR] 0-1:160) in CryptoPS T1-positive samples and 1:2560 (IQR 1:1280-1:10240) in T2-positives.

Conclusions: Diagnostic accuracy of the CryptoPS assay was sub-optimal in the context of CrAg screening, with poor sensitivity at low CrAg titers. However, the CryptoPS assay reliably detected individuals with high titers associated with poor outcomes.

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Introduction

Cryptococcal meningitis (CM) remains a major cause of mortality in persons living with HIV (PLWH), causing an estimated 15% of HIV-related deaths worldwide (1). In individuals with advanced HIV disease (AHD), detection of cryptococcal antigen (CrAg) in the blood has been shown to precede the development of CM (2, 3). CrAg screening in patients with AHD starting antiretroviral therapy (ART), with targeted pre-emptive fluconazole in CrAg-positives, has been shown to reduce incident CM and all-cause mortality (4), and is recommended by the World Health Organization (WHO) in those starting or reinitiating ART with a CD4 T-cell count <200 cells/µL (5).

Pre-emptive fluconazole therapy for CrAg-positive individuals reduces but does not completely eliminate excess mortality (6); up to one-third of asymptomatic CrAg-positive patients with AHD have disseminated central nervous system (CNS) disease and may require more potent antifungal regimens for effective treatment (5-7). Higher CrAg titers (>1:160) are associated with increased risk of CNS disease and death in CrAg-positive individuals (7-9). Titers can be assessed through serial dilution using commercial assays, but this requires extended labor and additional costs making it impractical in resource-limited settings. Semi-quantitative CrAg assays may provide a simpler means of stratifying risk for CrAg-positive patients with AHD and guiding differentiated treatment strategies.

CryptoPS (Biosynex, Strasbourg, France) is a semi-quantitative immunochromatographic assay that detects all *Cryptococcus neoformans/gattii* serotypes and provides results within 10 minutes. The assay has a control band, qualitative T1 band for CrAg-positivity, and semi-quantitative T2 band indicating a "high" CrAg titer (Figure 1). Just two limited diagnostic evaluations have been reported to date (10, 11). In a small study of patients with a CD4 <100 cells/µL in Cameroon, CryptoPS had good agreement with a commercial lateral flow assay (LFA) (IMMY diagnostics, Norman, Oklahoma, USA) and enzyme immunoassay (EIA) [Premier CrAg, Meridian Bioscience, Inc. Newtown, OH, USA], with a median reciprocal EIA titer >1:160 correlating with T2 band positivity; although the sample size which included

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just 14 serum CrAg-positive patients limited estimates of precision (10). A second study testing 99 archived serum samples selected from a cohort of HIV-positive Ugandans undergoing CrAg screening reported similar findings, with reasonable agreement between the CryptoPS and IMMY LFA and median CrAg titers of 1:320 in those with T2 band positivity (11); however, this study was also limited by a small sample size. CryptoPS was not tested on whole blood samples likely to be beneficial for point-of-care testing in either study; and the assay has not been evaluated prospectively and under routine care conditions.

We investigated diagnostic performance of the CryptoPS assay in a prospective CrAg screening cohort in Botswana, evaluating test accuracy (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV]) against a validated reference standard. To determine titer cutoffs for the CryptoPS T2 band (high-titer), we compared CryptoPS readings with IMMY CrAg titer results by serial dilution.

Materials and Methods

From January 2018 - January 2019, we performed real-time CrAg testing on EDTA whole blood samples from a sequential cohort of individuals with CD4 ≤200 cells/µL undergoing routine reflex CrAg screening at the Botswana-Harvard HIV Reference Laboratory (BHHRL). The BHHRL performs all CD4 testing for 27 ART clinics and a central referral hospital located around Gaborone. The full cohort has been described in detail elsewhere (12); briefly, CD4 T-cell count testing was performed using flow cytometry (FACSCalibur, Becton, Dickinson and Company, Franklin Lakes, NJ, USA); residual EDTA blood from sequential samples with a CD4 ≤200 cells/µL was screened for CrAg in real-time using the IMMY LFA (IMMY, Norman, OK, USA) as a validated reference standard (13, 14). CryptoPS testing was performed by a second trained operator blinded to IMMY LFA results. Results were interpreted by visual reading as negative (only control band visualized), T1 band positive (with control and T1 bands visualized by the

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operator), or T2 band positive (control, T1, and T2 bands visualized), as per manufacturer's instructions (Figure 1).

As the CryptoPS is not registered for diagnostic use in Botswana, all clinical management decisions were based on IMMY LFA results. IMMY LFA positive patients were managed according to the Botswana National HIV Management Guidelines (15). Individuals who tested positive by CryptoPS but negative by the IMMY LFA were contacted by the study team for re-testing by IMMY LFA and monitored over 3 months to evaluate for development of incident CM but did not receive antifungal therapy unless the repeat IMMY LFA results were positive. Three-month outcomes in these individuals with positive CryptoPS but negative IMMY LFA results were assessed through telephone contact, review of a national electronic medical records system, review of clinical notes, or further contact with clinical providers as needed.

At the end of the study period, IMMY LFA titers were determined by serial dilution of plasma samples stored at -80° Celsius. To enable further validation of the CryptoPS semi-quantitative results, we used both the CryptoPS and IMMY LFA to test an additional collection of 141 archived known CrAg-positive plasma samples from: 1) patients in a CrAg screening cohort from 2015-2016 (8); and 2) patients with CM enrolled in a completed phase II clinical trial evaluating induction therapies (16). All CrAg testing was performed by laboratory scientists blinded to previous testing results.

Finally, to better characterize samples with discordant CryptoPS and IMMY LFA test results, we re-tested all discordant samples with Meridian's CrAg EIA [Meridian Bioscience, Cincinnati, OH] and IMMY's EIA (IMMY, Norman, OK) at the National Institute for Communicable Diseases (NICD) in Johannesburg, South Africa. This study was approved by institutional review boards at the Botswana Ministry of Health and Wellness, the University of Botswana, Princess Marina Hospital, and the University of Pennsylvania.

Statistical analysis.

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In the prospective CrAg screening cohort, we determined the sensitivity, specificity, PPV, and NPV of the CryptoPS assay with 95% confidence intervals (95%CI) using the IMMY LFA assay as a reference standard. Using all CrAg-positive samples from the prospective screening cohort, plus the additional archived CrAg-positive samples, we evaluated the median and interquartile range of dilutional IMMY LFA CrAg titer values in samples with CryptoPS that were T1 band and T2 band positive, and displayed results graphically.

Laboratory findings, including the results of cryptococcal EIA testing, were described in samples with discordant CryptoPS/LFA results, and clinical outcomes reported in individuals with negative IMMY LFA but positive CryptoPS results. Analyses were performed using Stata Version 16 (College Station, TX, USA).

Results

Sensitivity and specificity of CryptoPS in a sequential cohort of individuals with CD4 ≤200 cells/µL

Nine-hundred and sixteen sequential blood samples were tested with the IMMY LFA and CryptoPS CrAg assays from 870 unique patients (46 had repeat CD4 testing and reflex CrAg screening, Table 1); median age was 39 years (interquartile range [IQR]: 33-46), 58.4% (507/868, 2 missing data) were male, and the median CD4 count was 142 cells/µL (IQR: 85-174 cells/µL). Using IMMY LFA testing, 41 of the 916 samples were CrAg-positive (4.5%, 95% confidence interval [CI]: 3.2-6.0%) with a median CrAg titer of 1:80 (IQR: 1:20 to 1:960). Using the IMMY LFA as the reference standard, the CryptoPS sensitivity was 61.0% (95%CI: 44.5-75.8%), and specificity was 96.6% (95%CI: 95.1-97.7%), yielding a PPV of 45.5% (95%CI: 32.0-59.4%) and NPV of 98.1% (95%CI: 97.0-98.9%) in our cohort (Table 2).

The CryptoPS assay was positive in 55/916 (6.0%, 95%CI: 4.6-7.7%) of samples; 25/55 (45.5%) were IMMY LFA positive and classified as “true positives” (18 T1 band and 7 T2 band). The remaining 30/55 (54.5%) CryptoPS positives were IMMY LFA negative and classified as “false-positives” (29 T1 band

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and 1 T2 band). On confirmatory testing, all 30 of the CryptoPS false-positives were Meridian EIA negative, and 29/30 were IMMY EIA negative, with one low-level positive (Figure 2a). Sixteen samples were IMMY LFA positive but CryptoPS-negative and classified as "false-negatives"; all had IMMY LFA CrAg titers ≤1:160, and 13 of the 16 had titers ≤1:40. Three of the sixteen were only LFA positive in the lowest dilution tested (1:2) and negative on the IMMY EIA (Figure 2b).

Detailed characteristics of the 30 false-positive CryptoPS results are shown in Table 3. The thirty "false-positive" CryptoPS samples were from 29 patients (one had two tests); 79% (23/29) received repeat IMMY LFA testing of whom 100% (23/23) were CrAg-negative. Most (97%, 28/29) were on ART at the time of CrAg screening and none received pre-emptive fluconazole. None (0/29) were diagnosed with cryptococcal meningitis in the 3 months following CD4 testing and reflex CrAg screening, and 28/29 were alive at completion of follow-up. One patient died 5 days after the date of CD4 testing after presenting to hospital with a clinical diagnosis of a lower respiratory tract infection; no central nervous system infection was suspected and the patient did not receive a lumbar puncture as part of his diagnostic evaluation. The patient's CryptoPS result was T1 band positive, with negative IMMY LFA, IMMY EIA, and Meridian EIA.

Given the relatively low sensitivity and specificity of the CryptoPS assay on EDTA whole blood, we repeated CryptoPS testing on frozen plasma samples after study completion blinded to previous results, which yielded very similar results to real-time whole blood testing for these specimens (Table S1). We also repeated the analysis restricted to individuals with CD4 cell counts ≤100 cells/μL; in this subgroup the sensitivity of the CryptoPS was 83.3% (95% CI: 58.6-96.4%) and specificity was 96.5% (95% CI: 93.5-98.4%) (Table S2).

Relationship between CryptoPS bands and dilutional CrAg titers

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182 Combining the samples from the sequential CrAg screening cohort with 141 archived IMMY LFA CrAg-
183 positive blood samples, 870 were CryptoPS negative, 102 samples were T1 band positive, and 84 were
184 T2 band positive (one CryptoPS T2 positive individual in the sequential cohort did not have sufficient
185 residual plasma for titration). The median IMMY LFA titer of the T1 band positive samples was 1:20 (IQR:
186 LFA negative to 1:160). Median titers in the CryptoPS T2 band positive samples were 1:2560 (IQR:
187 1:1280 to 1:10240); 79/84 (94%) of T2 positive samples had titers of ≥1:160 (Figure 3).
188
189 **Discussion**
190 In this prospective evaluation of the novel semi-quantitative CryptoPS CrAg LFA in HIV-seropositive
191 individuals with CD4 ≤200 cells/µl undergoing CrAg screening, we found sub-optimal assay sensitivity of
192 the CryptoPS assay compared to an established CrAg LFA (IMMY). The lower sensitivity of the CryptoPS
193 was exclusively due to false-negative test results on blood samples with low CrAg titers; all samples with
194 a titer of >1:160 were detected by the assay.
195 Our study adds to two prior small diagnostic validation studies of the CryptoPS assay. A study of
196 stored serum samples from Cameroon found a test sensitivity of 78% (11/14) against the IMMY LFA (10).
197 The median CD4 count of samples in this study was lower than in our cohort (44 cells/µL versus 142
198 cells/µL), likely resulting in a higher median CrAg titer (1:160 versus 1:80) and contributing to slightly
199 higher observed sensitivity (12). A second study of 99 cryopreserved serum samples from a Ugandan
200 CrAg screening cohort found a CryptoPS assay sensitivity of 88% (50/57, 95%CI: 76%-95%) and specificity
201 of 95% (40/42, 95%CI: 84%-99%) against the IMMY LFA; however, only 65% of samples with an IMMY
202 LFA dilutional titer of ≤1:20 were positive on the CryptoPS assay (11). Similarly, in our prospective
203 evaluation all (16/16) false-negative whole blood CryptoPS results were on samples with CrAg titers
204 ≤1:160, 13 (81%) with titers ≤1:40. These patients may be at relatively low risk for progression to

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meningitis in the absence of pre-emptive fluconazole, assuming prompt initiation or continuation of effective ART (17).

While the CryptoPS assay had sub-optimal overall sensitivity, the T2 band performed well in discriminating samples that had high CrAg titers with greater risk of meningitis and or failure of fluconazole pre-emptive therapy (6), with 79/84 (94%) of T2 band positive samples from stored blood found to have titers of ≥1:160 by serial dilution. The assay may have clinical utility in recognizing CrAg-positive patients who require further diagnostic evaluation for meningitis and enhanced antifungal treatment, albeit at the expense of missing a proportion of patients with lower CrAg titers who are still likely to benefit from standard pre-emptive fluconazole treatment (18). To avoid missing these individuals with lower titers, the CryptoPS could be used as a second test, performed after an initial IMMY LFA screen, to identify the highest risk individuals with T2 bands for enhanced care. The ease of use and interpretation of the CryptoPS tests may make this an attractive strategy in some settings.

We found moderate specificity of the CryptoPS assay, in keeping with the previous study from Uganda but with greater estimated precision given our larger sample size (11). We carefully followed the 29 patients with presumed false-positive CryptoPS results (positive CryptoPS result and negative IMMY LFA) for a period of at least 3 months. All patients (23/29) with repeat IMMY LFA testing again tested negative, all were negative with secondary testing using the Meridian EIA and all but one with the IMMY EIA. None developed meningitis despite receiving no antifungal treatment, supporting classification of these tests as false-positives. Further, nearly all were confirmed to be alive over follow-up; one patient died shortly after screening, but for causes likely unrelated to cryptococcal disease. Given a relatively low prevalence of cryptococcal antigenemia in patients with AHD (19), the positive predictive value of the CryptoPS assay in the prospective cohort was below 50%, with more false-positive than true positive results.

Our study is subject to several limitations. *Cryptococcus spp.* isolates have significant serotype diversity within Southern Africa, which may impact test sensitivity (20, 21). Although the CryptoPS assay can detect all *Cryptococcus neoformans/gattii* serotypes, different genotypic and phenotypic characteristics of local fungi could potentially have impacted test performance. Secondly, although IMMY LFA and CryptoPS tests were read by trained laboratory operators blinded to testing results on the other assay, we did not have two independent reviewers read the CryptoPS assay to evaluate inter-rater reliability. However, we repeated CryptoPS testing on the same stored plasma specimens with similar test results, and all IMMY LFA-positive samples underwent further testing through serial dilution confirming positive results, so findings are highly likely to be valid.

In summary, the CryptoPS assay provides rapid semi-quantitative CrAg test results on whole blood specimens and good discrimination for high-titer results. Limitations in sensitivity and specificity may limit its use clinically in cryptococcal antigen screening programs which typically aim to detect individuals with relatively low cryptococcal antigen burdens. These limitations may be less marked when testing individuals with suspected cryptococcal meningitis who have a higher pre-test probability of infection and higher blood CrAg titers.

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339 **Figure Legend**

340 **Figure 1. CryptoPS semi-quantitative assay.** 20 µL of blood specimen are added to the collection well of
341 the cassette followed by 3 drops of sample diluent with the result read at 10 minutes. A. Negative result
342 (control band positive); B. Low-titer positive (T1 and control bands positive); C. High-titer positive (T1, T2,
343 and control bands positive). Patient initials and study identification codes written on the bottoms of the
344 test strips have been concealed to ensure anonymity of the research participants.

345

346 **Figure 2. Discordant CryptoPS and IMMY LFA results.** (A) CryptoPS "false-positive" results with secondary
347 testing using the IMMY EIA; 29/30 samples tested negative on the IMMY EIA at an optical density below
348 positive cutoff and one sample tested positive at an optical density of 0.625. All samples tested negative
349 using the Meridian EIA. (B) CryptoPS "false-negative" results completed to IMMY LFA dilutional titer and
350 IMMY EIA optical density; 16/16 samples had an IMMY LFA titer of ≤1:160 and 3 tested negative on the
351 IMMY EIA at an optical density below positive cutoff, all 3 were IMMY LFA positive only in undiluted
352 samples indicating very low titers.

353

354 **Figure 3. Comparison of CryptoPS titer results and dilutional titers obtained using the IMMY LFA.** The
355 violin plots show the distribution of dilutional CrAg titers in CryptoPS negative, T1 (low titer), and T2 (high
356 titer) samples; 916 prospectively screened samples and 141 additional archived CrAg-positive plasma
357 samples. 25 CryptoPS negative samples (16 from the prospective cohort and 9 archived samples) were
358 positive on IMMY testing, all at titers of ≤1:160. 29 CryptoPS T1 positive and 1 CryptoPS T2 positive
359 samples were IMMY LFA negative. Median titers in the T1 and T2 groups with interquartile ranges are
360 shown on the figure. Note that in the treatment trial from which some of the archived samples were from
361 the maximum dilutional titer measured was 1:2560, hence some titers at this level may have been higher
362 if further dilutions were performed.

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370 **Figure 1.**
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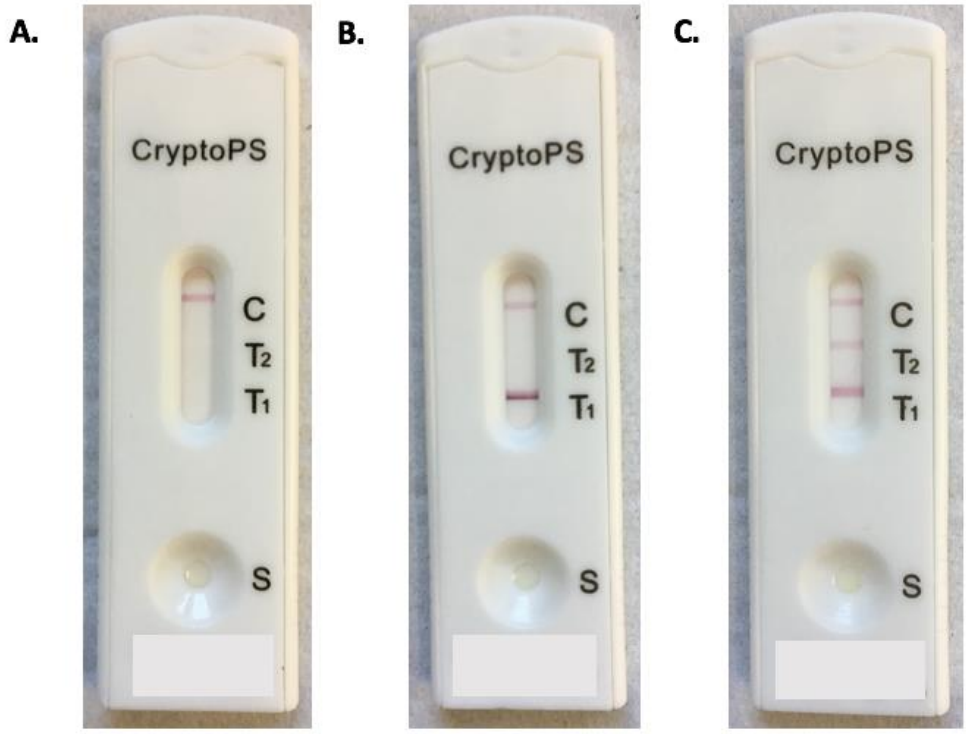

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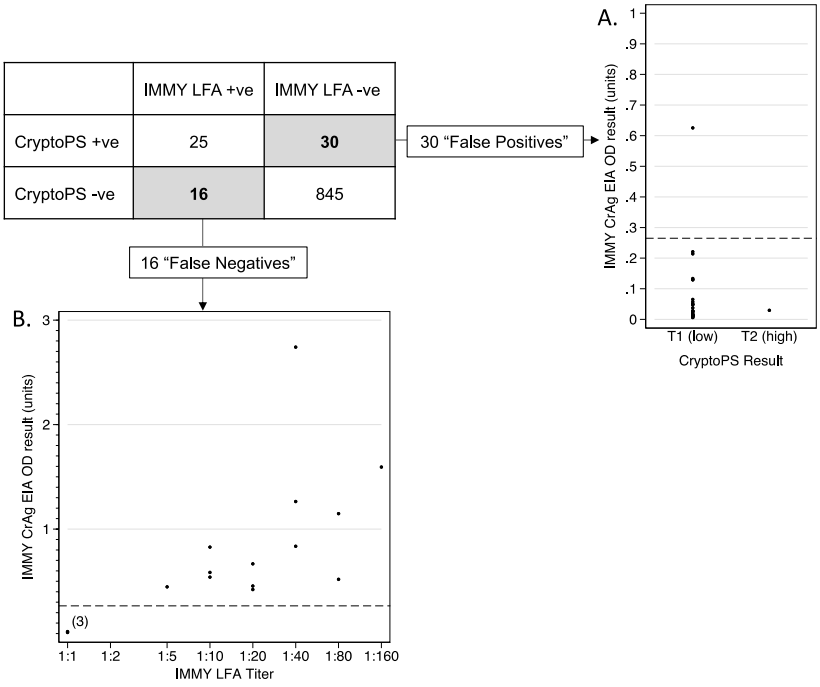
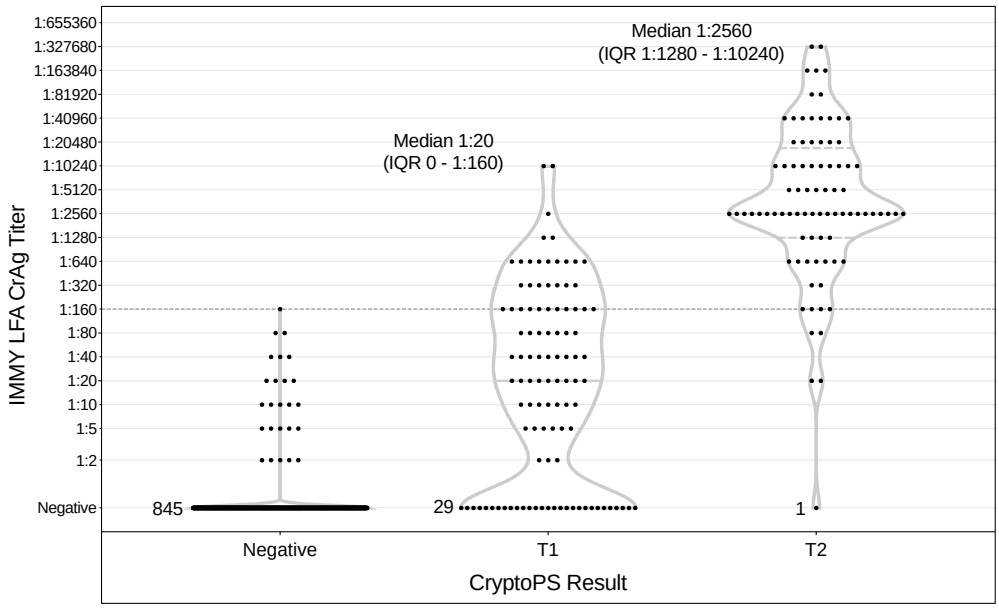
374 **Figure 2.**
375



376

Figure 3.



384 **Table 1.** Baseline characteristics of study participants

Variable	**Value** (median, IQR or %, n)
Sequential cohort of individuals with CD4 cell counts ≤200 cells/µL screened for CrAg (916 samples from 870 individuals)[*]	
Age (years)[†]	39 years (IQR 33-46)
Sex (% male)[†]	58% (507/868[†])
CD4 count (cells/µL)[§]	142 cells/µL (IQR 86-174)
Cryptococcal antigenemia[**] (% positive)[§]	4.5% (n=41)
ART status (% on ART)[§]	75% (n=691/916)
Prior cryptococcal meningitis (%)[†]	1% (n=13/870)
Additional individuals included in titer validation cohort (all CrAg-positive, n=141)[**]	
Age (years)	38 years (IQR 32-44)
Sex (% male)	58% (81/139[†])
CD4 count (cells/µL)	31 cells/µL (IQR 12-63)
Cryptococcal antigenemia[**] (% positive)	100% (n=141)

385 ART = antiretroviral therapy; CrAg = cryptococcal antigen; IQR = interquartile range; LFA = lateral flow assay
386 [*] 916 samples tests on 870 unique patients ; 46 individuals had repeat samples
387 [†] Age data were missing for 2 individuals. Age, sex, and prior cryptococcal meningitis data are reported for the 870
388 individuals in the CrAg screening cohort at the time of their first CrAg test
389 [§] Shows CD4 counts, IMMY CrAg LFA result, and ART status at the time of testing (some patients were tested twice
390 during screening period); of 226 patients not on ART at date of CD4 testing, 17% (39) had defaulted ART and 83%
391 (187) had no prior history of ART use
392 [**] CrAg results obtained using the IMMY CrAg lateral flow assay
393

394 **Table 2.** Sensitivity, specificity, positive, and negative predictive value of the CryptoPS semi-quantitative

395 assay versus the conventional IMMY LFA test, whole blood samples.

		IMMY LFA +ve	IMMY LFA -ve	Total
	CryptoPS +ve	25	30	55
	CryptoPS -ve	16	845	861
	Total	41	875	916
Sensitivity		61.0%	95% CI 44.5% - 75.8%	
Specificity		96.6%	95% CI 95.1% - 97.7%	
Positive predictive value		45.5%	95% CI 32.0% - 59.4%	
Negative predictive value		98.1%	95% CI 97.0% - 98.9%	

396 CI = confidence interval; LFA = lateral flow assay; SQ = semi-quantitative, +ve = positive; -ve = negative

397

398 **Table 3.** Clinical characteristics and outcomes of patients with false-positive CryptoPS tests on whole blood specimens.

Case	Age	Sex	CD4 count, cells/ µL	On ART	Prior CM	Repeat IMMY LFA	Received antifungal therapy	Incident CM within 3 months	Death within 3 months	Comments
1	53	Male	169	Yes	No	Negative	No	No	No	
2	65	Male	106	Yes	No	Negative	No	No	No	
3	40	Female	25	Yes	No	Negative	No	No	No	
4	48	Female	145	Yes	No	Negative	No	No	No	
5	50	Female	106	Yes	No	Negative	No	No	No	
6	24	Female	44	Yes	No	Negative	No	No	No	
7	31	Female	83	Yes	No	Negative	No	No	No	
8	37	Female	161	Yes	No	Negative	No	No	No	
9	41	Male	115	Yes	No	Negative	No	No	No	
10	7	Male	197	Yes	No	Negative	No	No	No	
11	19	Male	143	Yes	No	Negative	No	No	No	
12	43	Female	158	Yes	No	Negative	No	No	No	
13	41	Male	32	Yes	No	Negative	No	No	No	
14	19	Female	147	Yes	No	Negative	No	No	No	
15	24	Male	134	Yes	No	Negative	No	No	No	
16	32	Male	149	Yes	No	Negative	No	No	No	
17	36	Female	103	Yes	No	Negative	No	No	No	
18	48	Male	194	Yes	No	Negative	No	No	No	
19	42	Male	106	Yes	No	Negative	No	No	No	
20	32	Female	68	Yes	No	Negative	No	No	No	
21	52	Female	193	Yes	No	Negative	No	No	No	
22*	45	Male	98	Yes	No	Negative	No	No	No	Lumbar puncture and CSF testing for cryptococcus (by CSF India ink and culture) 31 days after HIV test date, all CSF tests negative
23	33	Male	86	Defaulted	No	Negative	No	No	No	Re-started ART, CD4 increased to 156 cells/µL and HIV viral load 652 copies/µL 133 days after first CD4 test.
24	39	Female	83	Yes	No	Not performed	No	No	No	Moved from Gaborone and unable to repeat IMMY LFA; re-established care in Gaborone with HIV-clinic visit 308 days after CrAg test, no cryptococcal meningitis diagnosed during follow-up.
25	39	Male	125	Yes	No	Not performed	No	No	No	Unable to contact for repeat CrAg testing; attended HIV-clinic visit 287 days after CrAg test, no cryptococcal meningitis diagnosed during follow-up.

26	20	Female	190	Yes	No	Not performed	No	No	No	Unable to contact for repeat CrAg testing; HIV-clinic visit 363 days after CrAg test, no cryptococcal meningitis diagnosed during follow-up.
27	unknown	Male	171	Yes	No	Not performed	No	No	No	Participant unable to meet with study team for re-testing but asymptomatic; repeat CD4 testing at 61 days from first test showed CD4 of 271 cells/μL and HIV viral load <400 copies/mL.
28	29	Female	125	Yes	No	Not performed	No	No	No	Unable to contact patient; seen in clinic 37 days after CD4 test date and was stable without reported symptoms and HIV viral load <400 copies/mL; no subsequent laboratory evaluation for CM or documentation of death from national death registry.
29	51	Male	32	Yes	No	Not performed	No	No	Yes	Presented to referral hospital Emergency department 5 days after CD4 and CrAg test date; vomiting, anorexia, wasting with dehydration. Admission diagnosis of pneumonia and/or pulmonary tuberculosis, anemia, and renal failure. Died the following day; no clinical evidence of meningitis and no lumbar puncture and CSF evaluation performed; unlikely to have died from CM.

399 ART = antiretroviral therapy; CrAg = cryptococcal antigen; CM = cryptococcal meningitis; CSF = cerebrospinal fluid; LFA = lateral flow assay

400 * Patient had two positive CryptoPS test results

401 See Supplementary Materials File for tables S1 and S2

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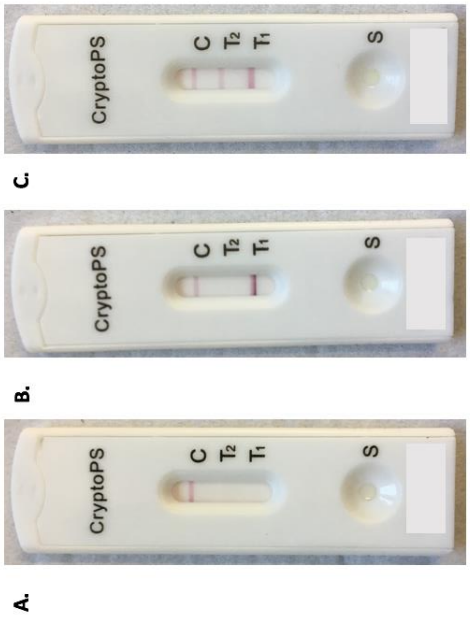

	IMMY LFA +ve	IMMY LFA -ve
CryptoPS +ve	25	**30**
CryptoPS -ve	**16**	845

30 “False Positives”

16 “False Negatives”

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