DIAGNOSTIC PERFORMANCE OF A SEMI-QUANTITATIVE POINT OF CARE ASSAY FOR CRYPTOCOCCOSIS

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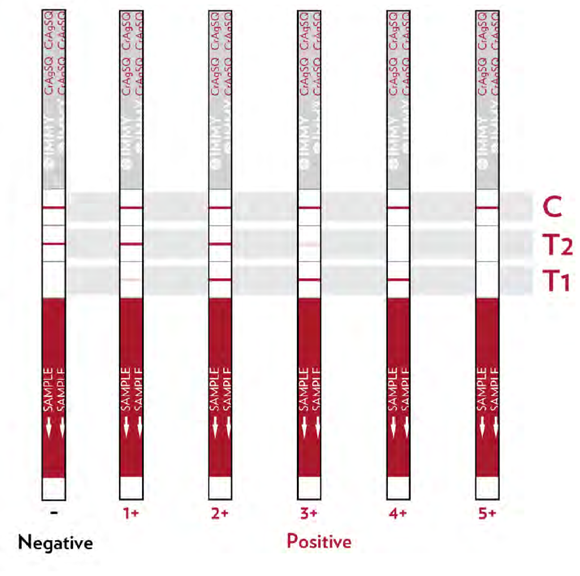
Introduction

- Cryptococcal antigen (CrAg) can be detected in the CSF and blood of persons with disseminated cryptococcal disease using a lateral flow assay (LFA).
- Studies have demonstrated that CrAg titer is predictive of meningitis and death [Letang 2015, Beyene 2017, Rajasingham 2019]. In particular, titers >1:160 have increasing probability of CSF involvement [Wake 2018].
- There is interest in a rapid, semi-quantitative test that can approximate titers at the bedside while maintaining the excellent sensitivity and specificity of the current reference standard LFA
- We describe the diagnostic performance of a novel all-in-one IMMY CrAg semi-quantitative (SQ) lateral flow assay (LFA) compared to the FDA-approved IMMY CrAg LFA in the rapid diagnosis of cryptococcosis.

Methods

- We compared the diagnostic performance of the CrAgSQ against the CrAg LFA in prospectively enrolled Ugandans with HIV-associated cryptococcal meningitis.
- CrAgSQ assay performance was determined on both cerebrospinal fluid (CSF) (n=93) and serum (n=83).
- The CrAgSQ grades (1+ to 5+) were compared with CrAg LFA titers to determine the approximate antigen burden each grade represents.
- CrAgSQ grades were plotted for 14-day survival.



The new semi-quantitative IMMY CrAgSQ demonstrated **excellent sensitivity** (100%) and **specificity** (100%) compared to CrAg LFA when tested on CSF and serum in HIV-associated cryptococcal meningitis.

Results

Table 2: CSF CrAgSQ grade compared to CSF titers, culture, and survival

CSF							
IMMY CrAgSQ CSF Grade	N	IMMY CrAg LFA Titer		Sterile Culture	Quantitative Culture *Cryptococcus* CFU / mL CSF		Survival 14-day
		Median	IQR		Median	IQR	
1+	2	1:5	--	100%	0	--	100%
2+	6	1:60	40 – 160	33%	60	0 – 32,000	100%
3+	16	1:7680	1920 – 10240	0%	4,700	530 – 120,000	93%
4+	39	1:40960	20480 – 163840	0%	110,000	27,000 – 370,000	81%
5+	2	1:327680	--	0%	9,600,000	--	50%

Table 3: Serum CrAgSQ grade compared to serum titers

Serum Grade	N	Median	Titer IQR
1+	1	1:5	--
2+	6	1:100	40 – 160
3+	13	1:5120	1280 – 10240
4+	30	1:40960	20480 – 81920
5+	2	1:1179648	--

Table 4: Contingency table of CrAgSQ against reference standard CrAg LFA in CSF

		IMMY CrAg LFA	
		Positive	Negative
IMMY CrAgSQ	Positive	65	0
	Negative	0	28

100% sensitivity (95%CI, 0.94–1.00); 100% specificity (95%CI, 0.88–1.00)

Baseline Characteristics

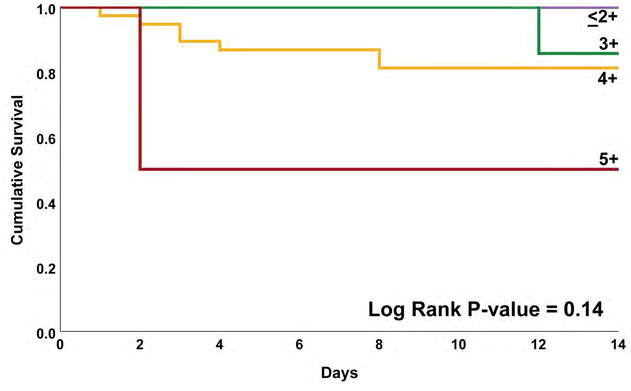
Table 1: Baseline demographics and lab parameters for those with tested CSF

Baseline Data	CrAg LFA Negative	CrAg LFA Positive	P-Value
N	28	65 [a]	--
Age, years	33 (27, 39)	34 (30, 45)	0.26
Men	21 (75%)	41 (63%)	0.26
Receiving ART [b]	9 (36%)	19 (29%)	0.53
WBCs ≥5 cells/μL	7 (26%)	19 (30%)	0.72
Protein, mg/dL	105 (39, 151)	69 (40, 128)	0.42

Values are median (IQR) or N (%); P-values calculated by Mann-Whitney U or chi-square.
[a] **One subject was not initially positive by CrAg LFA until dilution due to prozone.**
[b] Three comatose participants were missing ART data.

Survival Curve

Figure 1: 14-Day Survival Stratified by CSF CrAgSQ Grade



Conclusion

- The IMMY CrAgSQ demonstrated excellent sensitivity and specificity as compared with the FDA-approved reference standard CrAg LFA.
- The assay performed equally well on CSF and serum in HIV-infected persons with cryptococcal meningitis.
- The CrAgSQ has the additional advantage of detecting false negative results caused by the prozone effect.

Disclaimer: The authors have no conflicts of interests to declare. Specifically, there is no financial interest with the manufacturer or any royalties.

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