



Research Paper

A Phase III Diagnostic Accuracy Study of a Rapid Diagnostic Test for Diagnosis of Second-Stage Human African Trypanosomiasis in the Democratic Republic of the Congo



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ABSTRACT

Objectives: To estimate the diagnostic accuracy of HAT Sero K-SeT for the field diagnosis of second-stage human African trypanosomiasis (HAT).

Design: A phase III diagnostic accuracy design. Consecutive patients with symptoms clinically suggestive of HAT were prospectively enrolled. We compared results of the index test HAT Sero K-SeT with those of a composite reference standard: demonstration of trypanosomes in cerebrospinal fluid (CSF), or trypanosomes detected in any other body fluid AND white blood cell count in CSF >5/μL.

Setting: Rural hospital in the Democratic Republic of the Congo.

Participants: All patients above five years old presenting at Mosango hospital with a neurological problem of recent onset at the exclusion of trauma.

Interventions: n.a.

Main Outcome Measures: Sensitivity and specificity of HAT Sero K-SeT test.

Results: The sensitivity of the HAT Sero K-SeT was 8/8 or 100.0% (95% confidence interval: 67.6 to 100.0%) and the specificity was 258/266 or 97.0% (94.2% to 98.5%).

Conclusion: The high sensitivity of the HAT Sero K-SeT is in line with previously published estimates, though the sample of HAT cases in this study was small. The specificity estimate was very high and precise. This test, when negative, allows the clinician to rule out HAT in a clinical suspect in a hospital setting in this endemic region.

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1. Introduction

Human African trypanosomiasis (HAT), also known as sleeping sickness, is an infectious disease caused by the parasite *Trypanosoma brucei* (*T b gambiense* or *T b rhodesiense* which usually has a fatal outcome after two to three years if left untreated (Buscher et al., 2017; Sudarshi et al., 2014). The parasite is transmitted by tsetse flies that are only

present in a number of Sub-Saharan African countries. The West African variant caused by *T b gambiense* (g-HAT) is the most common form of the disease, accounting for over 95% of worldwide cases, and 90% of those are diagnosed in the Democratic Republic of the Congo (DRC) (Buscher et al., 2017; Lumbala et al., 2015). HAT evolves through two stages, the haemo-lymphatic stage, and the meningo-encephalitic stage. During the first stage, symptoms are not very specific and include fever, itching and joint pains. Later, in the second stage, more specific symptoms develop such as behavioural changes, sensory and motor disturbances, and alteration in the sleeping-waking cycle that is so typical for this disorder. Intramuscular pentamidine injections are the standard treatment for first-stage g-HAT; second-stage is typically treated with nifurtimox-eflornithine combination therapy (NECT), which requires one week of intravenous infusions of eflornithine and ten days of nifurtimox per os. Given the significant logistical and financial challenges related to NECT therapy and the fact that 14% of treated patients

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suffer major drug-related adverse events (Priotto et al., 2009), the diagnosis of g-HAT should be verified with a highly specific confirmatory test before treatment initiation.

Current testing algorithms for HAT are specifically designed for mass population screening by dedicated mobile teams. In this context, the majority of those tested are healthy individuals, and the HAT prevalence rate is typically below 1%, even in foci of active transmission. In the first step, a screening test is performed, and for many years the Card Agglutination Trypanosomiasis Test (CATT) has been used for this purpose (Magnus et al., 1978). The CATT is supplied as a multi-dose format that is adapted to processing large batches of samples and requires a cold chain. In a second step, a variable sequence of parasitological examinations, i.e., lymph node aspirate smear, thick blood film, capillary tube centrifugation (CTC), mini-anion exchange centrifugation technique (mAECT), and modified single centrifugation (MSC) is performed to isolate and identify trypanosomes in body fluids (Chappuis et al., 2005). None of these confirmatory tests have adequate sensitivity when done as single test (Lutumba et al., 2007), except for the mAECT in buffy coat (Camara et al., 2010). The format of these screening and confirmation tests is not well adapted for use in a primary care facility (Mitashi et al., 2014) and the diagnostic algorithm is far too complex for use in this setting.

More recently, Rapid Diagnostic Tests (RDT) have been developed for use with individual patients (Table 1). In 2012, the SD Bioline HAT (Standard Diagnostics, Korea), a lateral flow immunochromatographic test, partly based on the same native antigen as the CATT, was introduced (Bisser et al., 2016). The sensitivity and specificity of this RDT were 89.3% (95% confidence interval (CI) = 83.3–93.3) and 94.6% (95% CI = 94.2–94.9) respectively (Bisser et al., 2016). Another company developed two similar RDT formats based on the same native HAT antigens (HAT Sero K-SeT and HAT Sero-Strip, Coris BioConcept, Gembloux, Belgium), which showed promise in a phase I study (Buscher et al., 2013) (See Box 1). A phase II study achieved a sensitivity of 99% (95% CI: 95%–100%) and a specificity of 99% (95% CI: 97%–100%) for the HAT Sero K-SeT (Buscher et al., 2014). A real-time, long-term stability study showed that HAT Sero K-SeT remains stable for 25 months at 4 °C, 30 °C and 40 °C, and for at least nine months at 45 °C. The format of these RDT is appropriate for use in a primary health care facility and the available evidence described above suggests high diagnostic accuracy, similarly sensitive but slightly less specific than CATT (Lumbala et al., 2017).

However, there are some methodological concerns with the evidence obtained in phase II studies. The sensitivity and specificity estimates quoted above were obtained in groups of a priori well-characterized HAT cases and non-cases, and usually in study designs where the reference testing (parasitology) was only done if a serological

screening test (CATT) was positive. In the study by Bisser et al. (Bisser et al., 2016), e.g., the “non-cases” were healthy persons presenting for a mass screening campaign, who had a negative CATT test and for whom no further parasitology was done. This is a methodological shortcoming in diagnostic evaluation as reference testing should not be conditioned by the index test or closely correlated test. The sensitivity estimates of the RDT obtained with these conditional designs may be inflated, as they will miss the same cases as the CATT. Also, the specificity estimate obtained in these phase II designs may not be entirely representative of the clinical setting where patients attend with other infections that can lead to cross-reactions (Lijmer et al., 1999). Hence the need to evaluate this new RDT in a clinical setting on a non-biased series of patients who are all suspect for HAT. In this study, we assessed the diagnostic performance of the HAT Sero K-SeT by evaluating its ability to detect g-HAT in consecutive patients with neurological disorders attending Mosango hospital in the DRC.

2. Methods

2.1. Study Design

The study was embedded in a larger research project examining the etiology and outcome of neurological syndromes in a rural hospital in DRC, reported elsewhere (Mukendi et al., 2017). To evaluate the diagnostic performance of this new RDT in HAT, we used a phase III diagnostic accuracy design (Zhou et al., 2002), i.e., a prospective study among the target population: those patients who would be tested by this RDT in the future. We enrolled the study participants consecutively, based on the inclusion and exclusion criteria, and before obtaining any information on index test or reference standard. The study complied with STARD recommendations for reporting diagnostic accuracy studies (Bossuyt et al., 2003), (Bossuyt et al., 2015).

2.2. Participants

We conducted the study at a 351-bed rural hospital in the Province of Bandundu (now Kwilu Province), DRC: the Hôpital Général de Référence de Mosango. Patients presenting with the following clinical symptoms and signs, (henceforward called *clinical suspects*), were prospectively recruited. All patients above five years of age presenting at Mosango hospital with ongoing or recent onset (less than two weeks) of any of the following neurological symptoms were eligible for enrollment: altered state of consciousness, changes in sleep pattern, cognitive decline, changes in personality or behavior, epileptic seizure(s), daily, severe or progressive headaches, meningism, cranial nerve lesions,

Table 1
Summary of published evidence on diagnostic accuracy of two Rapid Diagnostic Tests for HAT.

| | Sensitivity | | Specificity | |
|---------------------------------|-------------------------------|--|-------------------------------|--|
| | Prototype SD Bioline HAT | HAT Sero K-SeT | Prototype SD Bioline HAT | HAT Sero K-SeT |
| Phase I Proof of concept | 0.82 (0.81–0.83) ^b | 0.94 (0.88–1.00) ^a | 0.97 (0.96–0.98) ^b | 0.99 (0.97–1.00) ^a |
| Phase II On archived samples | 1.00 (0.98–1.00) ^d | 0.99 (0.95–1.00) ^c 0.99 (0.97–1.00) ^d | 0.88 (0.83–0.92) ^d | 0.99 (0.97–0.99) ^c 0.88 (0.84–0.92) ^d |
| Phase III Prospective cohort | | | | |
| In screening campaign | 0.88 (0.81–0.93) ^e | | 0.94 (0.94–0.95) ^e | |
| In health center | 0.93 (0.80–0.97) ^e | | 0.98 (0.97–0.98) ^e | |

^a Büscher P, Gillemann Q, Lejon V. Rapid diagnostic test for sleeping sickness. *N Engl J Med*. 2013 Mar 14;368 (11):1069–70.

^b Sternberg JM, Gierliński M, Biéler S, Ferguson MA, Ndung'u JM. Evaluation of the diagnostic accuracy of prototype rapid tests for human African trypanosomiasis. *PLoS Negl Trop Dis*. 2014 Dec 18;8 (12):e3373.

^c Büscher P, Mertens P, Leclipteux T, Gillemann Q, Jacquet D, Mumba-Ngoyi D, Pyana PP, Boelaert M, Lejon V. Sensitivity and specificity of HAT Sero K-SeT, a rapid diagnostic test for serodiagnosis of sleeping sickness caused by *Trypanosoma brucei gambiense*: a case-control study. *Lancet Glob Health*. 2014 Jun;2 (6):e359–63.

^d Jamonneau V, Camara O, Ilboudo H, Peyllhard M, Koffi M, Sakande H, N'Dri L, Sanou D, Dama E, Camara M, Lejon V. Accuracy of individual rapid tests for serodiagnosis of gambiense sleeping sickness in West Africa. *PLoS Negl Trop Dis*. 2015 Feb 2;9 (2):e0003480.

^e Bisser S, Lumbala C, Nguertoum E, Kande V, Flevaud L, Vatunga G, Boelaert M, Büscher P, Josenando T, Bessell PR, Biéler S, Ndung'u JM. Sensitivity and Specificity of a Prototype Rapid Diagnostic Test for the Detection of *Trypanosoma brucei gambiense* Infection: A Multi-centric Prospective Study. *PLoS Negl Trop Dis*. 2016 Apr 8;10 (4):e0004608.

Box 1

Definitions of phases 1, 2, and 3 for diagnostic studies (Boelaert et al., 2007).

Phase 1: The aim of phase 1, early exploratory studies, is to provide proof-of-principle that the marker is strongly associated with the disease of interest. This requires a relatively small number (10–100) of samples, usually archived specimens.

Phase 2: The candidate test is evaluated in a case-control design, on several series of subjects (or archived samples of subjects), who are enrolled on the basis of their disease status. The sample size should be a minimum of 100 subjects in each series. The control subjects should be of different kinds: healthy non-endemic, healthy endemic and patients with potentially cross-reacting diseases.

Phase 3: Phase 3 studies are large-scale prospective studies validating the test in the target population, requiring a sufficiently large (usually minimum 300) and representative sample of consecutively enrolled or randomly selected patients. This design yields the best estimate of specificity because non-cases are people with other, potentially cross-reacting diseases.

Phase 3-FIELD: Phase 3-FIELD studies are completed in field conditions by representative health personnel, such as mid-level health workers in a clinic, rather than specialized laboratory technologists in a reference laboratory.

sensory-motor deficits or other focal neurological signs, or gait disorders. We excluded those unwilling or unable to give written informed consent (either directly or via proxy); to comply with the study requirements; and those with neurological symptoms clearly related to recent trauma, or sequelae of previous well-established neurological events (e.g., stroke).

2.3. Test Methods

2.3.1. Clinical and Laboratory Assessment

The study neurologist assessed each enrolled patient clinically and also through the use of a pre-established set of laboratory tests including RDTs and reference assays (either done on-site or in reference laboratories in Kinshasa, DRC or Antwerp, Belgium) (Barbe et al., 2016). Response to specific treatment was also assessed, and patients were followed up for a further six months following discharge. All patients were tested with the RDT (or index test) as well as the reference tests, on admission. The index RDTs were performed first and interpreted by the laboratory technician before he read the reference test result. A photograph was taken of each RDT read-out, for later quality assurance. Appropriate pre-study training in *Good Clinical and Laboratory Practices* and regular supervision by clinical monitors ensured that all requisite standards were met and all procedures were harmonized (Ravinetto et al., 2015).

2.3.2. Index Tests

Each kit of the HAT Sero K-Set RDT (Coris, BioConcept, Gembloux, Belgium) mentioned recommended storage conditions, expiry date, and batch number. On the study site, the kits were kept at the recommended storage conditions. Temperature and humidity of the storage locations were electronically logged for quality assurance purposes. Batch changes were closely monitored. The test procedures used were in compliance with the instructions for use. In brief, for the HAT Sero K-Set, the individually sealed package was opened, and the presence of two green lines in the reading window was verified. The cassette was then partially pushed back into the packaging, to cover the reading window. The technician transferred 30 μ l of venous heparinized blood to the specimen well in the cassette using a micropipette. He then

added two drops of buffer solution, pushed the cassette fully back into the packaging and waited 15 min. The test run was only considered valid when a red line was observed in the control line position. Valid test runs were only read as positive if a red line (even if very faint) was observed in the T (for 'test') position. A photograph was taken for later verification.

We also performed CATT on every patient as it is the recommended *standard of care* for the diagnostic identification of a suspected HAT case, and the results are suitable for comparison with other RDT outcomes. CATT was carried out in accordance with the manufacturer's instructions (Magnus et al., 1978) using one drop of whole blood. A pictorial was used as a reference for a semi-quantitative read-out of the strength of agglutination. The technician read the agglutination reaction as positive if agglutination was very strong (+++), strong (++), moderate (+), or weak (+/-).

2.3.3. Reference Standard

We used a composite reference standard for defining a second-stage g-HAT case: demonstration of trypanosomes in cerebrospinal fluid (CSF), or if BOTH of the following conditions were met: trypanosomes detected in any other body fluid AND white blood cell count in CSF > 5/ μ l. In CSF, modified single centrifugation (MSC) was done, a technique with >80% sensitivity (Büscher et al., 2009; Miezán et al., 2000). The techniques to demonstrate trypanosomes in any other body fluid included cervical lymph node aspirate (LNA) if typical ganglions were present, and blood using the mini-anion exchange centrifugation technique (mAECT). A more detailed description of these parasitology techniques and all the standard operating procedures used in the laboratory are publicly available elsewhere (Barbe et al., 2016).

2.4. Analysis

We calculated the *sensitivity* of the index test based on the percentage of those testing positive in the group of true second-stage g-HAT cases, as defined by the reference standard. We calculated the *specificity* of the RDT as the proportion of subjects testing negative in the group not suffering from second-stage g-HAT according to our reference standard. We estimated the 95% confidence interval using Wilson's score method for binomial proportions. We excluded subjects with missing results for the index test or reference standard from the analysis.

As the target product profile for the RDT under evaluation requires *sensitivity* and *specificity* levels of at least 90% and a required precision of the estimates set at +/- 5.9%, these assumptions led to a minimum requirement of 100 cases of second-stage g-HAT and as many patients with an alternative diagnosis. Anticipating a prevalence rate (*prior probability*) of second-stage g-HAT of 20% in this series of clinical suspects, the required sample size was projected at 500 patients with neurological disorders, to be recruited over an 18 months period.

2.5. Ethical Aspects

The Institutional Review Board of the University Hospital Antwerp, Belgium, and the Ethical Committee of the School of Public Health in Kinshasa approved the protocol. After giving their free, informed consent to participate, all patients were examined by a neurologist, and, based on his initial assessment, treatment was offered free-of-charge. If the patient was confirmed as a HAT case, he/she was treated according to the national guidelines issued by the national control program (PNLTHA).

3. Results

Between September 2012 and January 2015, we studied a total of 351 patients ranging from 6 to 78 years with a mean age of 39 years, of whom 163/351 (46.4%) were male. Table 2 shows the demographic and clinical characteristics of the study population. On admission, 16% of the patients presented with a known chronic comorbidity such as

Table 2
Demographic and clinical characteristics of the study population (n = 351).

| Patient characteristics ^a | n (%) |
|--------------------------------------|-------------|
| Male sex | 163 (46.4%) |
| Mean age, years (standard deviation) | 39.1 (17.7) |
| Minimum – maximum age, years | 6–78 |
| Known comorbidity | 57 (16.2%) |
| - Diabetes mellitus | 11 (3.1%) |
| - Hypertension | 32 (9.1%) |
| - Other cardiovascular problems | 3 (0.9%) |
| - Other conditions | 11 (3.1%) |
| Medicines taken before admission | 149 (42.5%) |
| - Antibiotics only | 42 (12.0%) |
| - Antimalarials only | 28 (8.0%) |
| - Both antibiotics and antimalarials | 52 (14.8%) |
| - Other | 27 (7.7%) |

^a More details on the clinical profile and outcomes of this case series have been reported elsewhere (Mukendi et al., 2017).

hypertension, other cardiovascular problems, or diabetes mellitus. Thirty-five percent (122/351) had taken antimalarials, antibiotics, or both, before admission. More details on the clinical profile and outcomes of the full case series have been reported elsewhere (Mukendi et al., 2017).

Though the reference tests were completed as soon as possible after admission since they formed the basis for treatment decisions, one eligible patient died almost immediately upon enrollment, and therefore no diagnostic evaluation was possible. Full reference standard data was not available for 60 patients because lumbar puncture was contra-indicated (n = 10), refused (n = 9), unsuccessful (n = 24) or because modified single centrifugation results were missing (n = 17) (Fig. 1). None of these 60 patients had a positive parasitology result

for HAT in the mAECT done on blood samples. The reference standard for second-stage HAT done on CSF confirmed ten out of the remaining 290 (3%) patients as HAT cases. In the non-HAT patients, infectious diseases were diagnosed at frequencies ranging from 1 to 5%: unspecified meningoencephalitis (5%); bacterial meningitis (4%); malaria (4%), Pott's disease (2.5%), HIV/AIDS (3%), central nervous system tuberculosis (1%) and tetanus (1%).

Out of a total of 351 eligible study subjects, 16 did not receive the HAT Sero K-SeT RDT, as it was out-of-stock at one point, and, as mentioned above, one patient died before he could be tested. In total, 274 patients were included in the main analysis. The median number of white blood cells (WBC) per μl cerebrospinal fluid (CSF) was 1 (interquartile range 1–3). Forty-three patients (16%) had >5 WBC/ μl CSF. The eight patients classified as cases of second-stage g-HAT in this study all had >5 WBC/ μl CSF. Their values ranged between 23 and 1030 WBC/ μl CSF with a median of 270 and an interquartile range of 57–320 WBC/ μl CSF. The eight second-stage g-HAT cases all had positive parasitological test results in CSF. The results of all index and reference diagnostic tests for these eight patients are given in Table 3. Table 4 compares the neurological presentation in the group of eight included HAT cases with those of the non-cases.

Fig. 1 and Table 5 show the results we obtained when comparing the HAT Sero K-SeT with the reference standard (n = 274). There were no invalid test results, nor indeterminate results, but there were six faint test lines out of 17 positive tests (35.3%). The sensitivity of the RDT was 8/8 or 100.0% (95% CI 67.6 to 100.0) and the specificity 258/266 or 97.0% (95% CI 94.2 to 98.5). The two true HAT cases that were not tested with the HAT Sero K-SeT in Mosango, but were tested at a later stage at ITM-Antwerp, were also found to be positive (results not included in sensitivity estimate). Therefore all ten, true HAT cases were eventually proven positive with the index RDT. Considering a *pre-test*

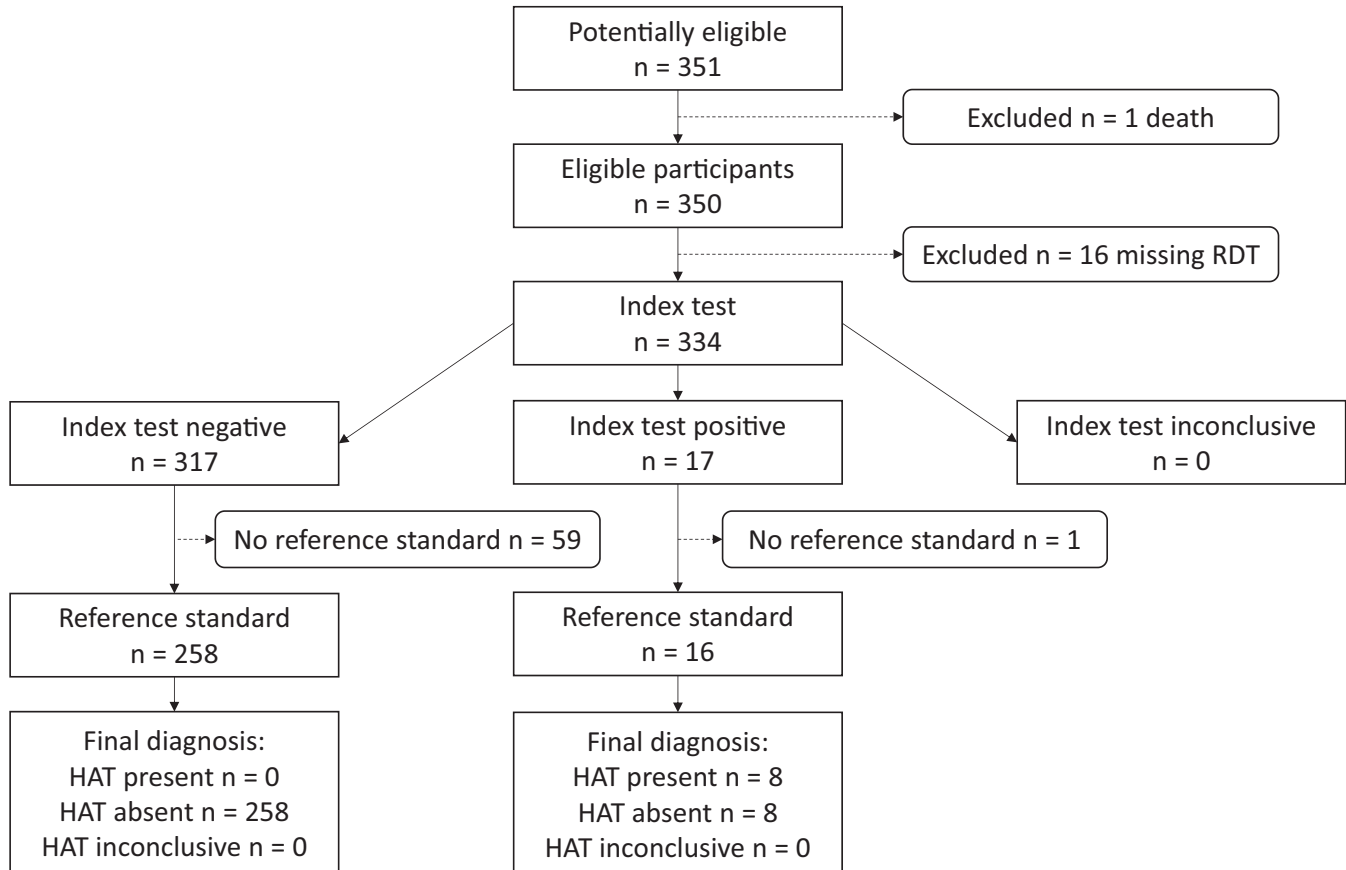


Fig. 1. Patient flow diagram.

Table 3

Results of diagnostic tests targeting g-HAT in cases with second-stage g-HAT, n = 8, included in the analysis, and two other cases for whom no RDT result was available.

| Patient | Trypanosomes in CSF (modified single centrifugation) | Trypanosomes in cervical lymph node aspirate | mAECT in blood | WBC count per μ l CSF | HAT Sero K-SeT RDT | CATT whole blood |
|---------|--|--|----------------|---------------------------|--------------------|------------------|
| 1 | Positive | Not done | Negative | 320 | Positive | Positive |
| 2 | Positive | Not done | Negative | 320 | Positive | Positive |
| 3 | Positive | Not done | Positive | 1030 | Positive | Positive |
| 4 | Positive | Not done | Positive | 220 | Positive | Positive |
| 5 | Positive | Positive | Positive | 320 | Positive | Positive |
| 6 | Positive | Positive | Positive | 23 | Positive | Positive |
| 7 | Positive | Not done | Positive | 68 | Positive | Positive |
| 8 | Positive | Not done | Negative | 24 | Positive | Positive |
| 9 | Positive | Not done | Positive | 47 | Not done | Positive |
| 10 | Positive | Not done | Not done | 74 | Not done | Positive |

CATT: Card Agglutination Test for Trypanosomiasis; CSF: cerebrospinal fluid; mAECT: mini-anion exchange centrifugation technique; RDT: rapid diagnostic test; WBC: white blood cells.

probability of 3% (10/351) in the study population, the *positive predictive value* was 8/16 or 50.0% (95% CI 26.6 to 73.4), and the *negative predictive value* was 258/258 or 100.0% (95% CI 98.5 to 100.0). *Post-test probability* dropped to zero when the test was negative, firmly ruling out HAT in this setting.

Table 6 shows the performance of the CATT in the 287 samples for which both CATT and reference standard results were available. The *sensitivity* of the CATT was 10/10 or 100.0% (95% CI 72.3 to 100.0) and the *specificity* 268/277 or 96.8% (95% CI 93.9 to 98.3). The *positive predictive value* was 10/19 or 52.6% (95% CI 31.7 to 72.7), and the *negative predictive value* was 268/268 or 100.0% (95% CI 98.6 to 100.0). CATT results were comparable to those of the HAT Sero K-SeT as shown in Table 7.

Frequently, patients were classified as extremely unwell on admission, and we observed an overall case fatality rate during this study of 8%. Twenty-two percent of the survivors developed a severe, post-disease disability. We observed no adverse events related to any of the diagnostic procedures.

4. Discussion

The diagnostic accuracy of RDT HAT Sero K-SeT was comparable to that of CATT, but RDT was easier to perform. The main strength of our study lay in its phase III design as this is much more representative of the clinical setting that exists in rural hospitals in HAT endemic areas. This design challenges the specificity estimates that were obtained from previous laboratory-based studies, since, in a laboratory setting, the archived samples used as controls are not representative of the clinical setting.

Previous studies evaluating tests carried out on well-characterized cases plus a separate control group (two-gate, case-control or phase II

Table 4

Distribution of neurological symptoms and signs that were used as inclusion criteria in study, in patients with and without second-stage g-HAT included in the analysis, n = 274.

| Neurological symptoms& signs | Total, n = 274 n (%) | 2nd stage g-HAT, n = 8 n (%) | Non-cases, n = 266 n (%) |
|--|----------------------|------------------------------|--------------------------|
| Daily, severe or progressive headaches | 129 (47) | 2 (25) | 127 (48) |
| Meningism | 88 (32) | 2 (25) | 86 (32) |
| Gait disorders | 72 (26) | 5 (63) | 67 (25) |
| Epileptic seizure(s) | 68 (25) | 0 (0) | 68 (26) |
| Sensory-motor deficit or other focal signs | 59 (22) | 2 (25) | 57 (21) |
| Changes in personality or behavior | 52 (19) | 6 (75) | 46 (17) |
| Changes in sleep pattern | 44 (16) | 6 (75) | 38 (14) |
| Altered state of consciousness | 39 (14) | 1 (13) | 38 (14) |
| Cranial nerve lesions | 19 (7) | 0 (0) | 19 (7) |
| Cognitive decline | 13 (5) | 1 (13) | 12 (5) |

g-HAT: human African trypanosomiasis caused by *Trypanosoma brucei gambiense*.

Table 5

Diagnostic performance of the HAT Sero K-SeT versus reference standard^a (n = 274)^b.

| Sero K-SeT | Reference standard | | Total |
|------------|--------------------|----------------|-------|
| | Positive (n,%) | Negative (n,%) | |
| Positive | 8 (100.0%) | 8 (3.0%) | 16 |
| Negative | 0 (0.0%) | 258 (97.0%) | 258 |
| Total | 8 (100.0%) | 266 (100.0%) | 274 |

^a Reference standard: the presence of trypanosomes in cerebrospinal fluid, OR trypanosomes detected in any other body fluid AND white blood cell count in CSF > 5/ μ l.

^b 13 samples could not be tested in Mosango because of stock-out of RDT during the study period.

studies) tend to overestimate the diagnostic performance compared with studies undertaken in a clinical population (Lijmer et al., 1999). Another strength of this study and a major difference with other studies is that the index test reading and the parasitology result were obtained in an independent way, as enrollment and performing the reference standard was not conditioned by any prior serological test. Previous field evaluations of RDTs for HAT have been mainly done within a study population with at least one positive serological screening test (usually the CATT), and only in case of a positive CATT test, parasitological examinations for the confirmation of HAT were done. All persons without this first positive screening test are considered non-HAT cases by default in such designs. While this serological pre-screening allows recruiting more true HAT patients in a shorter time, it inherently tends to overestimate sensitivity, as the false-negative errors of serological tests are most likely correlated. In the present study, there is absolutely no bias towards the presence of antibodies and therefore no bias towards over-estimated sensitivity.

On the other hand, the main limitations of our study were the low number of true HAT cases that were enrolled (10 out of 351), leading to imprecise estimates of sensitivity, and the fact that we could not obtain a reference standard result in a substantial number of patients. The sensitivity estimates were, nonetheless, very consistent with those obtained in earlier studies, and the specificity estimate was sufficiently precise. The non-availability of the reference standard was, for the large part, statistically random, and we expect, therefore, little bias in the estimates. We should, of course, remain conscious of the fact that our reference standard was not perfect, as its sensitivity cannot be considered optimal, even when employing this combination of parasitological tests (Lutumba et al., 2007). By any means, such deficit in sensitivity may have affected the specificity estimate of the index test, resulting in an underestimation (Boelaert et al., 1999; Büscher et al., 2013), which does not seem to have been the case here. We acknowledge that this study was limited to stage 2 HAT only, as it was embedded in a larger study on the neurological syndrome, and stage 1 patients have none or few, non-specific, signs and symptoms. Nonetheless, we believe our data are relevant to clinical practice.

Overall, the HAT Sero K-SeT was easy to use. One, potentially confusing step was the need to check for the presence of a green test indicator as well as a control line before the application of the sample. Also, the requirement to partially push back the RDT in the packaging during the test was somewhat unusual and slightly cumbersome. Our study

Table 6

Diagnostic performance of CATT versus reference standard (n = 287).

| CATT | Reference standard ^a | | Total |
|----------|---------------------------------|----------------|-------|
| | Positive (n,%) | Negative (n,%) | |
| Positive | 10 (100.0%) | 9 (3.0%) | 19 |
| Negative | 0 (0.0%) | 268 (97.0%) | 268 |
| Total | 10 (100.0%) | 277 (100.0%) | 287 |

CATT: Card Agglutination Test for Human Trypanosomiasis.

^a Reference standard: the presence of trypanosomes in cerebrospinal fluid, OR trypanosomes detected in any other body fluid AND white blood cell count in CSF > 5/ μ l.

Table 7
Comparison of diagnostic accuracy of HAT Sero K-SeT and CATT.

| Parameter | | HAT Sero K-SeT % [95% CI] | CATT % [95% CI] |
|---------------------------|-----------------|------------------------------|-------------------------|
| Prevalence | Pr(HAT) | 2.9% [1.3% to 5.67%] | 2.9% [1.3% to 5.7%] |
| Sensitivity | Pr(RDT + HAT) | 100% [63.1% to 100%] | 100% [72.3% to 100.0%] |
| Specificity | Pr(RDT- No HAT) | 97% [94.2% to 98.7%] | 96.8% [93.9% to 98.3%] |
| Positive predictive value | Pr(HAT RDT +) | 50% [24.7% to 75.3%] | 52.6% [31.7 to 72.7%] |
| Negative predictive value | Pr(No HAT RDT-) | 100% [98.6% to 100%] | 100.0% [98.6 to 100.0%] |

Pr = probability; RDT = Rapid Diagnostic Test; HAT = human African Trypanosomiasis.

corroborates, to a great extent, the findings of Büscher et al. in early-stage development, as well as those of Bisser et al. obtained with the SD Bionline (Bisser et al., 2016). Within the clinical settings of rural DRC, a patient presenting at a hospital with an ongoing or recent neurological episode who tests negative for the HAT based on Sero K-SeT is almost certainly not suffering from sleeping sickness, and, therefore other aetiologies should be considered. The *likelihood ratio* of a positive HAT Sero K-SeT test result is 33.3. The *likelihood ratio* indicates the extent to which a positive HAT Sero K-SeT result changes the probability that a patient with clinical symptoms and signs suggestive of HAT, truly suffers from second-stage g-HAT. In this study population, prior to testing, the odds of second-stage g-HAT (the pre-test odds) is 0.03 (8/266). The post-test odds of second-stage g-HAT can be calculated as the pre-test odds multiplied by the likelihood ratio. For a positive test result, this formula gives 0.03×33.25 or 1.0. This means that a positive Sero K-SeT result is a fairly strong argument in support of a diagnosis of second-stage g-HAT. A positive Sero K-SeT result raises pre-test probability from 2.9% to a post-test probability figure of 50.0%; the test is therefore remarkably accurate in predicting the diagnosis of second-stage g-HAT. However, national guidelines require confirmation by parasitology as current treatment is not safe enough to be given to non-HAT cases. The *likelihood ratio* of a negative HAT Sero K-SeT test result is 0. Therefore, a negative test result turns a pre-test odds of second-stage g-HAT of 0.03 into a post-test odds of 0 (= pre-test odds * likelihood ratio = 0.03×0). This means that a negative Sero K-SeT advances a very strong argument against a diagnosis of second-stage g-HAT; the test is, therefore, excellent for ruling out the diagnosis of second-stage g-HAT. No further parasitological testing is needed in these circumstances.

As the production cost of the HAT Sero K-SeT is currently above 1€/unit, its price may limit large-scale deployment in central Africa. The company is currently developing a second-generation RDT test kit replacing native with recombinant antigens and attempting to reduce the unit costs.

In conclusion, this study supports the previously reported high sensitivity of the HAT Sero K-SeT and confirms a very high specificity among disease suspects in field conditions. A negative HAT Sero K-SeT allows health workers to rule out sleeping sickness in patients presenting with a new onset neurological episode. A positive test allows selecting the patients with neurological disorders who should be referred to facilities where extensive parasitological workup is available. This RDT is a promising new tool for clinical use as its format is more appropriate for primary care settings than CATT while its accuracy is similar.

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Marleen Boelaert (corresponding author) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Registration

The study protocol was registered in the [ClinicalTrials.gov](https://clinicaltrials.gov) database with Identifier: NCT01589289 where it can be accessed at <https://clinicaltrials.gov/ct2/show/NCT01589289>

Data Sharing

Anonymized patient-level data are available on request from the corresponding author at epaessens@itg.be. Individual consent for data sharing was not obtained but the presented data are anonymized, and risk of identification is low.

Authors' Contributions

PL, FC, CY, EB, DM, and MB drafted the study protocol. PG, BB, LM, JRK, EB, and DB conducted the data collection under the supervision of PL, BB, EB, and MB. DM, KV, and MB analyzed the data. DM and MB wrote the first draft of the paper, and all authors participated in its critical review. All authors endorse the final version and approve the submission.

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