Performance of clinical signs and symptoms, rapid and laboratory diagnostic tests for diagnosis of human African trypanosomiasis by passive screening in Guinea: a non-interventional, prospective cross-sectional study

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Research Article

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Abstract

Background: Passive diagnosis of human African trypanosomiasis (HAT) at the health facility level is a major component of HAT control in Guinea. We examined which clinical signs and symptoms are associated with HAT, and assessed the performance of selected clinical presentations, of rapid diagnostic tests (RDT), and of laboratory tests on dried blood spots (DBS) for diagnosing HAT.

Method: The study took place in 11 health facilities in Guinea, where 2345 clinical suspects were tested with RDTs HAT Sero-K-Set, rHAT Sero-Strip, and SD Bioline HAT. Seropositives underwent parasitological examination to confirm HAT and their DBS were tested in indirect ELISA/ T.b. gambiense, trypanolysis, LAMP and m18S qPCR. Multivariable regression analysis assessed association of clinical presentation with HAT. Sensitivity, specificity, positive and negative predictive values of key clinical presentations, of the RDTs and of the DBS tests for HAT diagnosis were determined.

Results: The HAT prevalence, as confirmed parasitologically, was 2.0% (1.5-2.7%). Odds ratios (OR) for HAT were increased for participants with swollen lymph nodes (OR 96.7), important weight loss (OR 20.4), severe itching (OR 45.9) or motor disorders (OR 4.5). Presence of at least one of these clinical presentations was 75.6% (73.8-77.4%) specific and 97.9% (88.9-99.9%) sensitive for HAT. HAT Sero-K-Set, rHAT Sero-Strip, and SD Bioline HAT were respectively 97.5% (96.8-98.1%), 99.4% (99.0-99.7%) and 97.9% (97.2-98.4%) specific, and 100% (92.5-100.0%), 59.6% (44.3-73.3%) and 93.8% (82.8-98.7%) sensitive for HAT. All DBS tests had specificities ≥ 92.9%. While LAMP and m18S qPCR sensitivities were below 50%, trypanolysis and ELISA/ T.b. gambiense had sensitivities of 85.3% (68.9-95.0%) and 67.6% (49.5-82.6%).

Conclusions: Presence of swollen lymph nodes, important weight loss, severe itching or motor disorders are simple but accurate clinical criteria for HAT referral in Guinea. Diagnostic performances of HAT Sero-K-Set and SD Bioline HAT are sufficient for referring positives to microscopy. Trypanolysis on DBS may discriminate HAT patients from false RDT positives.

Trial registration: The trial was registered under NCT03356665 in clinicaltrials.gov (November 29, 2017, retrospectively registered https://clinicaltrials.gov/ct2/show/NCT03356665).

Background

Infection with the parasite Trypanosoma brucei gambiense (T.b. gambiense) causes the chronic form of human African trypanosomiasis (HAT), also called sleeping sickness. While in Central Africa, the Democratic Republic of the Congo is responsible for about three quarters of all reported gambiense HAT patients, in West Africa, Guinea is frontrunner in the number of cases [1]. Almost all Guinean HAT cases occur along the coastline, in particular in the prefectures of Boffa, Dubreka and Forecariah [2, 3].

Despite considerable challenges, Guinea implements an efficacious HAT control program based on medical interventions supplemented with vector control. Even during the Ebola epidemic outbreak in 2014–2016, the national HAT control program managed to deploy insecticide impregnated targets in
Boffa, and limited parasite transmission to humans by reducing the tsetse fly vector density [4]. Medical interventions against HAT in Guinea consist of passive and active screening, followed by treatment of confirmed HAT cases. During active screening, a specialized team visits the most affected villages and tests the whole population. An important drawback however is that once the disease prevalence drops, cost-effectiveness of active screening decreases as fewer cases are detected [5]. Also, as experienced in Guinea in the recent past, during epidemics of other infections, active screening may be interrupted [6, 7]. While approaching the status of elimination of HAT as a public health problem, passive screening for HAT, integrated in the existing health system, therefore increases in importance, is more resilient to interruption and more sustainable. In Guinea, passive screening was maintained at a low level during the Ebola epidemic, and was rapidly resumed in 2016 [3]. In passive screening, serological testing for HAT among individuals consulting a health centre, is initiated by the observation of symptoms or signs considered “suggestive” for HAT [3]. In such clinical suspects, an antibody detection test, usually a rapid diagnostic test (RDT), is carried out. Subjects that are RDT positive are subsequently examined microscopically for trypanosome presence, while those that test RDT negative, are considered HAT free. As neither the RDT positive predictive values (PPV) nor the sensitivities of parasite detection techniques are 100%, not all RDT positive suspects are parasitologically confirmed. To further discriminate potential HAT patients from RDT false positives and better target additional labour intensive microscopic re-examinations, further laboratory tests can be carried out remotely on dried blood spots (DBS).

While the most sensitive parasite detection techniques are routinely applied in Guinea [3, 8], for nearly all the other steps of the diagnostic chain, different options to increase or decrease suspicion for HAT are available, which, depending on their HAT diagnostic performance, influence effectiveness of screening. Although the clinical picture of gambiense HAT is relatively well documented [9, 10], the association of signs and symptoms with HAT in a health care seeking population has hardly been studied [11]. Furthermore, in the last decade, several RDTs have emerged for individual screening of HAT clinical suspects [12, 13]. For DBS testing, trypanolysis and ELISA/ T. b. gambiense are available to detect antibodies against T. b. gambiense [14–16], while Trypanozoon specific DNA can be detected using the Loopamp Trypanosoma brucei Detection kit (LAMP) or m18S quantitative PCR (qPCR) [17, 18]. So far, for Guinea, the diagnostic performance of SD Bioline HAT, HAT Sero-K-Set and trypanolysis has mainly been evaluated retrospectively on stored plasma specimens or DBS [14, 19].

Within the framework of a multi-country diagnostic trial, the diagnostic performance of clinical signs and symptoms, of 3 RDTs and of serological and molecular laboratory tests on DBS was evaluated prospectively for diagnosis of HAT, in the context of passive screening in Guinea.

Methods

Study setting

In Guinea, clinical suspects were recruited for the DiTECT-HAT-WP2 study between January 2017 and January 2020 in 11 hospitals and health posts in the prefectures of Boffa, Dubreka and Forecariah. In
these 3 prefectures, the HAT prevalence expressed as number of HAT cases per 10000 inhabitants in 2017 was respectively 2.92, 0.53 and 1.51, and decreased to 0.97, 0.33 and 0.99 in 2019 [3]. Serological screening sites (SSS) offered clinical and serological screening for HAT, and referred participants who were RDT positive to a centre for diagnosis and treatment (CDT). The CDT performed parasitological examination of RDT positives, in addition to clinical and serological screening. In Boffa prefecture, participants were recruited in Boffa hospital that acted as the CDT, while the health posts of Soubouyadi, Tamita and Walia acted as serological screening sites. In Dubreka prefecture, Dubreka LTO was the CDT, with 2 SSS, the health centres of Dubreka CU and Kholoya. In Forecariah prefecture, the health centre of Karakoro acted as CDT, while the health centers of Benty, Konta, Madinagbe, M’Boro and Sinkinet were SSS.

**Study protocol**

The study protocol is summarized in Fig. 1. Individuals consulting the study SSS or CDT could be included if they had visited or resided in a HAT endemic area and presented with clinical suspicion for HAT. Clinical suspicion was defined as presence of at least one of the following clinical signs or symptoms: Recurrent fever not responding to anti-malarial medication; headache for a long duration (>14 days); presence of enlarged lymph nodes in the neck; important weight loss; weakness; severe itching; amenorrhea, abortion, or sterility; coma; psychiatric problems (e.g. aggressiveness, apathy, mental confusion, increasing unusual hilarity, etc.); sleep disruption (nocturnal insomnia and/or excessive diurnal sleeping); motor disorders (convulsions, abnormal movements, shaking, walking difficulties); or speech disorders. Individuals were excluded from participation if they had already been treated for HAT, did not give their written informed consent or were less than 4 years old.

Finger prick blood from clinical suspects participating in the study was tested with 3 RDTs, HAT Sero-K-Set (Coris Bioconcept, Belgium), rHAT Sero-Strip (Coris Bioconcept, Belgium) and SD Bioline HAT (Abbott, South Korea) according to the instructions of the manufacturers. Clinical suspects negative in all 3 RDTs were considered HAT free, while those that were positive in at least one RDT, were considered serological suspects. Parasitological examination of serological suspects was carried out in the CDTs, which in case of referral also repeated the RDTs (only RDT positives in the CDT were retained). If enlarged lymph nodes were present in the serological suspect, a lymph node puncture was performed and a drop of lymph was microscopically examined under 10x40 magnification for presence of trypanosomes. From every serological suspect, venous blood on heparin was taken. If no lymphadenopathy was present or no parasites had been observed in lymph, 4 ml of heparinized blood was centrifuged, theuffy coat was taken and analysed for presence of trypanosomes using the mini anion exchange centrifugation technique onuffy coat (mAECT-BC)[8]. For every serological suspect two types of DBS were prepared. On a Whatman grade 4 filter paper, 16 drops of 30 µl of heparinized blood were deposited and left to dry. In parallel, 180 µls of heparinized blood were lysed for 5 minutes with 20 µls of 5% SDS solution (Sigma Aldrich), and 2 drops of 40 µls of lysed blood were deposited on a Whatman grade 1001 filter paper. Filter papers were dried, packed in separate envelopes, which in turn were packed in hermetic plastic bags containing silicagel. A lumbar puncture was carried out on parasitologically confirmed HAT patients, or if
the clinician considered it appropriate, based on strong clinical suspicion. The cerebrospinal fluid (CSF) was examined for the number of white blood cells, and for presence of trypanosomes using the modified single centrifugation [20]. Patients with parasitologically confirmed HAT without CSF trypanosomes and white blood cell numbers ≤ 5/µl, were considered in first stage and HAT patients with > 5 white blood cells/µl or trypanosomes in CSF were classified in second stage. Treatment of HAT was carried out according to the treatment protocols in place at the CDTs. Serological suspects that could not be confirmed at the first microscopic examination, were invited for re-examination at the CDT or were re-examined by the national program. A number of RDT seropositives detected at SSS level, who didn’t show up at CDT were offered microscopic examination by the national sleeping sickness program (PNLTHA).

**Laboratory tests**

The DBS were sent to the Centre International de Recherche-Développement sur l’Elevage en zone Subhumide (Bobo-Dioulasso, Burkina Faso), where laboratory tests were performed. On DBS collected on Whatman grade 4 paper, trypanolysis and ELISA/ *T. b. gambiense* were carried out for *T. b. gambiense* specific antibody detection, both targeting LiTat 1.3 and 1.5 VSG, following the methodology previously described [21]. For *Trypanozoon* DNA detection, m18S qPCR was carried out on DBS collected on Whatman grade 4 and if positive, followed by TgsGp-qPCR, while the lysed blood collected on Whatman grade 1001 was tested with Loopamp *Trypanosoma brucei* Detection Kit (LAMP, Eiken Chemical, Tokyo, Japan), according to published methodologies [18].

**Data analysis**

Results obtained at the CDT were immediately entered in a digital case report form [22]. The application incorporated demographic, clinical and diagnostic data and including pictures or positive RDTs and videos of trypanosome positive microscopy results. Results from SSS were collected on a paper case report form and entered retrospectively in the application. Data were transferred from the application to a central database, and exported into a Microsoft Excel sheet. Descriptive statistics were carried out to check for missing data and variation in each variable; categorical variables were summarized as proportions, while continuous variables were summarized with the median value and range.

Regression analysis and evaluation of the diagnostic performance were based on the participants HAT status (Fig. 1). Participants with trypanosomes detected in lymph, blood or CSF were considered HAT positive. Participants who were triple RDT negative were considered HAT negative. Participants who were RDT positive, but in whom no trypanosomes could be detected after microscopic examination(s), were considered HAT negative. Participants who were RDT positive, but did not undergo any parasitological examination were disregarded.

Regression analysis using Stata Statistical Software (Release 14, College Station, TX: StataCorp LP) was performed to assess for associations with the HAT status. Continuous variables were assessed for normal distribution, and the correlation between the thirteen clinical signs and symptoms was determined. Unconditional associations between HAT status and the explanatory variables (gender, age, and clinical signs and symptoms) were investigated. Subsequently, mixed logistic regression models
were developed for the HAT status, with prefecture included as a random effect to account for spatial clustering within each prefecture. Backward elimination was then used to screen variables, and only statistically significant variables ($p \leq 0.05$) were retained. Two-way interaction terms between all remaining variables were assessed for statistical significance. The final multivariable model included variables that were either statistically significant, or were part of a significant interaction term [23]. The intra-cluster coefficient was computed as the proportion of overall variation due to variation between groups, while interaction terms were interpreted using the coefficients [24]. As an example, the odds of a patient being HAT positive when having both enlarged lymph nodes and itching (compared to a patient who had neither enlarged lymph nodes nor itching), was determined as follows: \( \text{exp} [\text{Coefficient Enlarged lymph nodes} + \text{Coefficient itching} + \text{Coefficient Enlarged lymph nodes*itching}] \).

The diagnostic performance of the clinical presentation (only those that were retained in mixed logistic regression), the three RDTs (individually, in parallel, and in series), and of the four laboratory tests was determined. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for diagnosis of HAT were calculated with 95% Clopper Pearson confidence intervals (GraphPad Prism 9). The Kappa agreement for combinations of RDTs and laboratory tests was also determined, and interpreted as poor (< 0.00), slight (0.00-0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80), or almost perfect (0.81-1.00) [25].

**Results**

**Descriptive statistics of field results**

In total, 2353 clinical suspects were included: 707 in the prefecture of Boffa, 705 in Dubreka, and 941 in Forecariah. Of these clinical suspects, 1320 (56.1%) were female and 1033 (43.9%) male. Their median age was 30 years (range: 4–89). The most frequently observed clinical presentations were fever (96%) and headache (80.3%), followed by weakness (21.1%) (Table 1). Overall, among the 2353 study participants (Fig. 1), 122 tested positive to at least one RDT (5.2%; 95% CI: 4.3–6.2%). Specifically, 110/2352 (4.7%; 95% CI: 3.9–5.6%) were positive to HAT Sero-K-Set; 44/2350 (1.9%, 95% CI:1.4–2.5%) were positive to rHAT Sero-Strip; and 100/2343 (4.3%, 95% CI: 3.5–5.2) were positive to SD Bioline HAT. Among the 122 RDT positives, 114 serological suspects were parasitologically examined (6 were lost to follow-up and 2 died before parasitology could be carried out). Forty-eight individuals were diagnosed with parasitologically confirmed HAT (48/2345, 2.0%; 95% CI: 1.5–2.7), among whom 26 were trypanosome positive in lymph (26/48, 54.2%; 95% CI: 39.2–68.6), and 28 in mAECT-BC (28/48, 58.3%; 95% CI: 43.2–72.4). Out of 21 RDT positives for whom both lymph and blood were examined, eight tested positive in both body fluids. In MSC, 18/40 HAT patients tested had trypanosomes in CSF (45.0%; 95% CI: 29.3–61.5), of which 2 had not been previously confirmed in lymph or blood (2/48, 4.2%; 95% CI: 0.5–14.3). The median CSF white blood cell count was 145/µl (range: 12-1086/µl). All HAT patients for whom CSF data were available (46/48) were in 2nd stage. The HAT patients included 19 females (39.6%) and 29 males (60.4%), and their median age was 26 years (range: 10–65).
Table 1

Frequency of clinical presentations in study participants and HAT patients, and association to HAT positivity. This table shows gender, age and the frequency of clinical symptoms and signs in clinical suspects and in HAT patients. The association to HAT positivity was assessed in univariable analysis and after multi-variable mixed logistic regression. OR: odds ratio; CI: confidence interval; * only females considered, the numbers used to estimate the frequency are shown in the brackets.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All participants N = 2353</th>
<th>HAT N = 48</th>
<th>Univariable analysis</th>
<th>OR (95% CI)</th>
<th>p</th>
<th>Multivariable mixed logistic regression</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>43.9% (1033)</td>
<td>60.4% (29)</td>
<td>0.02</td>
<td>1.96 (1.1–3.5)</td>
<td>0.016</td>
<td>3.1 (1.23–7.62)</td>
<td></td>
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<tr>
<td>Age</td>
<td>NA</td>
<td>NA</td>
<td>0.02</td>
<td>0.98 (0.96–1.00)</td>
<td>0.016</td>
<td>3.1 (1.23–7.62)</td>
<td></td>
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<tr>
<td>Fever</td>
<td>96.0% (2258)</td>
<td>89.6% (43)</td>
<td>0.03</td>
<td>0.36 (0.14–0.93)</td>
<td>&lt;0.001</td>
<td>3.1 (1.23–7.62)</td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>80.3% (1889)</td>
<td>91.7% (44)</td>
<td>0.06</td>
<td>2.73 (0.07–7.7)</td>
<td>&lt;0.001</td>
<td>3.1 (1.23–7.62)</td>
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<tr>
<td>Weakness</td>
<td>21.1% (496)</td>
<td>54.2% (26)</td>
<td>&lt;0.001</td>
<td>4.61 (2.6–8.2)</td>
<td>0.07</td>
<td>3.1 (1.23–7.62)</td>
<td></td>
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<tr>
<td>Weight loss</td>
<td>13.7% (323)</td>
<td>81.3% (39)</td>
<td>&lt;0.001</td>
<td>34.3 (16.3–72.6)</td>
<td>&lt;0.001</td>
<td>3.1 (1.23–7.62)</td>
<td></td>
</tr>
<tr>
<td>Sleep disruption</td>
<td>9.7% (229)</td>
<td>29.2% (14)</td>
<td>&lt;0.001</td>
<td>4.0 (2.1–7.6)</td>
<td>&lt;0.001</td>
<td>3.1 (1.23–7.62)</td>
<td></td>
</tr>
<tr>
<td>Enlarged lymph nodes</td>
<td>8.1% (190)</td>
<td>77.1% (37)</td>
<td>&lt;0.001</td>
<td>47.8 (23.9–95.6)</td>
<td>&lt;0.001</td>
<td>3.1 (1.23–7.62)</td>
<td></td>
</tr>
<tr>
<td>Itching</td>
<td>6.4% (150)</td>
<td>50.0% (24)</td>
<td>&lt;0.001</td>
<td>18.1 (9.8–33.3)</td>
<td>&lt;0.001</td>
<td>3.1 (1.23–7.62)</td>
<td></td>
</tr>
<tr>
<td>Amenorrhea*</td>
<td>6.1% (81/1320)</td>
<td>57.9% (11/19)</td>
<td>&lt;0.001</td>
<td>24.04 (9.4–61.7)</td>
<td>&lt;0.001</td>
<td>3.1 (1.23–7.62)</td>
<td></td>
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<tr>
<td>Motor disorders</td>
<td>4.1% (97)</td>
<td>52.1% (25)</td>
<td>&lt;0.001</td>
<td>36.2 (19.2–68.2)</td>
<td>0.07</td>
<td>3.1 (1.23–7.62)</td>
<td></td>
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</table>
### HAT status of study participants

For further analysis of the results, study participants were considered as true HAT positives if they were confirmed as HAT patients based on trypanosome observation during microscopy performed on blood, lymph or CSF specimens (n = 48). Clinical suspects were considered as true HAT negatives (n = 2297) if they (i) tested negative to all 3 RDTs (n = 2231); or (ii) were RDT positive, but parasite negative in microscopy (n = 66). The latter group included subjects (Fig. 1) who had all 4 laboratory tests negative (n = 37); subjects that did not undergo laboratory tests (n = 23); subjects that were laboratory test positive but in whom, upon re-examination in microscopy, no parasites could be found (n = 4); and subjects that were laboratory test positive but died before a second parasitological tests could be carried out (n = 2). The 8 RDT positives who were completely lost to follow-up and did not undergo any parasitology were excluded from further analyses.

### Clinical symptoms and signs associated with HAT, regression analysis

The frequency of the different inclusion clinical symptoms and signs, in HAT (n = 48) and non-HAT affected study participants (n = 2297), is summarized in Fig. 2.

Convulsions were highly correlated with coma (ρ = 0.87) and motor disorders (ρ = 0.63). Coma was also correlated with motor disorders (ρ = 0.70) and enlarged lymph nodes (ρ = 0.61). The results of the unconditional associations between the explanatory variables gender, age and clinical parameters, and the dependent variable HAT positivity, are presented in Table 1. While amenorrhea was univariably associated with HAT positivity, it was not included in the multivariable model since it only related to female participants. The final multivariable logistic regression model for HAT patients included five explanatory variables (gender, enlarged lymph nodes, weight loss, itching, and motor disorders) and two significant interaction terms (enlarged lymph nodes*itching and weight loss*motor disorders). Clinical suspects presenting with enlarged lymph nodes had the highest odds (96.74) to have HAT, followed by

<table>
<thead>
<tr>
<th></th>
<th>All participants N = 2353</th>
<th>HAT N = 48</th>
<th>Univariable analysis</th>
<th>Multivariable mixed logistic regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychiatric problems</td>
<td>1.9% (45)</td>
<td>22.9% (11)</td>
<td>&lt; 0.001</td>
<td>20.3, (9.5–43.4)</td>
</tr>
<tr>
<td>Speech disorders</td>
<td>1.2% (27)</td>
<td>0.0% (0)</td>
<td>0.98</td>
<td>-</td>
</tr>
<tr>
<td>Convulsions</td>
<td>0.8% (18)</td>
<td>10.4% (5)</td>
<td>&lt; 0.001</td>
<td>20.7, (7.0–61.0)</td>
</tr>
<tr>
<td>Coma</td>
<td>0.2% (4)</td>
<td>2.1% (1)</td>
<td>0.02</td>
<td>15.7, (1.59–156)</td>
</tr>
</tbody>
</table>
those that presented with itching or important weight loss. Males were at higher odds to have HAT than females. Odds of clinical suspects presenting with both enlarged lymph nodes and itching (compared to a participant who had neither enlarged lymph nodes nor itching) increased to 413 ($p = 0.03$). Similarly, when both weight loss and motor disorders were present (compared to a participant who had neither), the odds of being HAT positive increased to 2220 ($p = 0.01$). The intra-cluster correlation coefficient was $5.26 \times 10^{-12}$, suggesting that spatial clustering with prefecture was negligible.

**Diagnostic performance of clinical presentation**

The diagnostic performance of (co-)occurrence of the 4 clinical symptoms and signs that were associated singly or in combination with HAT, namely enlarged lymph nodes, itching, weight loss and motor disorders, was studied in function of the HAT status in 48 HAT and 2297 non-HAT affected study participants (Table 2). Presence of enlarged lymph nodes, and/or weight loss and/or itching and/or motor disorders had 97.9% sensitivity (only 1/42 HAT patients did not have one of these 4 symptoms or signs) and 75.6% specificity. Although the PPV of observing at least one symptom or sign remained limited to 7.7%, this increased to 39.3% when co-existence of 2 or more of the 4 selected clinical presentations were considered.

### Table 2

**The diagnostic performance of occurrence of 4 key clinical presentations for HAT diagnosis.** Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of occurrence of presence of enlarged lymph nodes and/or weight loss and/or itching, and/or motor disorders were determined for identification of HAT patients. The occurrence of at least one symptom or sign ($\geq 1/4$) and co-occurrence ($\geq 2/4$; $\geq 3/4$ or all $4/4$) of the 4 selected clinical presentations was counted in 48 HAT patients and 2297 non-HAT affected study participants and proportions (n/N) with 95% confidence intervals (CI) were determined.

<table>
<thead>
<tr>
<th>Number of signs or symptoms present</th>
<th>% Sensitivity (n/N, 95% CI)</th>
<th>% Specificity (n/N, 95% CI)</th>
<th>% PPV (n/N, 95% CI)</th>
<th>% NPV (n/N, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\geq 1/4$</td>
<td>97.9 (47/48, 88.9–99.9)</td>
<td>75.6 (1737/2297, 73.8–77.4)</td>
<td>7.7 (47/607, 5.7–10.2)</td>
<td>99.9 (1737/1738, 99.7–100.0)</td>
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<tr>
<td>$\geq 2/4$</td>
<td>87.5 (42/48, 74.8–95.3)</td>
<td>97.2 (2232/2297, 96.4–97.8)</td>
<td>39.3 (42/107, 30.05–49.2)</td>
<td>99.7 (2232/2238, 99.4–99.9)</td>
</tr>
<tr>
<td>$\geq 3/4$</td>
<td>56.3 (27/48, 41.2–70.5)</td>
<td>99.7 (2289/2297, 99.3–99.9)</td>
<td>77.1 (27/35, 59.9–89.6)</td>
<td>99.1 (2298/2310, 98.6–99.4)</td>
</tr>
<tr>
<td>$4/4$</td>
<td>18.8 (9/48, 9.0–32.6)</td>
<td>100 (2297/2297, 99.8–100)</td>
<td>100 (9/9, 66.4–100)</td>
<td>98.3 (2297/2336, 97.7–98.8)</td>
</tr>
</tbody>
</table>

**Diagnostic performance of rapid diagnostic tests**
For estimation of the RDT diagnostic performance in 48 HAT and 2297 non-HAT clinical suspects, a few participants had partially missing RDT results (Table 3, Fig. 3). Of the three RDTs, HAT Sero-K-Set had the highest sensitivity (100%), followed by SD Bioline HAT (93.8%) and rHAT Sero-Strip (59.6%). The highest specificity was observed with rHAT Sero-Strip (99.4%), while HAT Sero-K-Set and SD Bioline HAT had similar specificities of 97.5% and 97.9% respectively. The PPV of the individual RDTs ranged between 45.2% and 66.7%, while the NPV was between 99.2% and 100%. Using the RDTs in parallel resulted in a high sensitivity (93.8–100%), specificity (97.1–97.7%) and NPV (99.9–100%), while the PPV was limited (42.1–46.4%). In series combinations including rHAT Sero-Strip led to low sensitivities (59.6%), except for the HAT Sero-K-Set and SD Bioline HAT combination (93.6%). There was moderate agreement between HAT Sero-K-Set and rHAT Sero-Strip (Kappa = 0.52; SE = 0.02), and rHAT Sero-Strip and SD Bioline HAT (Kappa = 0.55; SE = 0.02), while the agreement between HAT Sero-K-Set and SD Bioline HAT was almost perfect (Kappa = 0.86; SE = 0.02).

Table 3

The diagnostic performance of 3 rapid diagnostic tests for diagnosis of HAT. The individual diagnostic sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

<table>
<thead>
<tr>
<th></th>
<th>% Sensitivity (n/N, 95% CI)</th>
<th>% Specificity (n/N, 95% CI)</th>
<th>% PPV (n/N, 95% CI)</th>
<th>% NPV (n/N, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAT Sero-K-Set</td>
<td>100.0 (47/47, 92.5–100.0)</td>
<td>97.5 (2240/2297, 96.8–98.1)</td>
<td>45.2 (47/104, 35.4–55.3)</td>
<td>100.0 (2240/2240, 99.8–100.0)</td>
</tr>
<tr>
<td>rHAT Sero-Strip</td>
<td>59.6 (28/47, 44.3–73.3)</td>
<td>99.4 (2281/2295, 99.0–99.7)</td>
<td>66.7 (28/42, 50.5–80.4)</td>
<td>99.2 (2281/2300, 98.7–99.5)</td>
</tr>
<tr>
<td>SD Bioline HAT</td>
<td>93.8 (45/48, 82.8–98.7)</td>
<td>97.9 (2239/2287, 97.2–98.4)</td>
<td>48.4 (45/93, 37.9–59.0)</td>
<td>99.9 (2239/2242, 99.6–100.0)</td>
</tr>
</tbody>
</table>

Diagnostic performance of laboratory tests on dried blood spots

Among the 48 HAT patients, 34/48 had a DBS and all 4 DBS test results were available for 33/34, while 1/34 HAT patient missed a LAMP result. Among the 66 RDT positive HAT negatives (Fig. 1), 43/66 had DBS and all 4 DBS test results were available for 42/43, while 1/43 missed a trypanolysis result. The individual results of all DBS are shown in Fig. 4.

Table 4 summarizes the diagnostic performance of each individual laboratory test. Trypanolysis (in parallel on *Trypanosoma brucei gambiense* variable antigen type LiTat 1.3 and LiTat 1.5) had the highest sensitivity (85.3%), followed by ELISA/ *T.b. gambiense* (67.6%). Sensitivities for m18S qPCR and LAMP were low. The highest specificity was observed for m18S qPCR (97.7%), followed by ELISA/ *T.b. gambiense* (95.3%). The PPV ranged between 80.0% for LAMP and 93.8% for m18S qPCR, while the NPV
ranged between 65.6% for LAMP and 88.6% for trypanolysis. There was fair agreement between LAMP and the three other laboratory tests (with trypanolysis: Kappa = 0.23; SE = 0.10; with m18S qPCR: Kappa = 0.25; SE = 0.11; with ELISA: Kappa = 0.29; SE = 0.11). There was moderate agreement between m18S qPCR and both trypanolysis (Kappa = 0.42; SE = 0.10) and ELISA/ T.b.gambiense (Kappa = 0.51; SE = 0.11). The agreement between ELISA/ T.b.gambiense and trypanolysis was almost perfect (Kappa = 0.81; SE = 0.11).

The TgsGp-qPCR was carried out on 19 DBS only, 13 from HAT patients and 6 from RDT positive HAT negatives. Among the tested HAT patients, TgsGp-qPCR sensitivity was 38.5% (5/13, 95% CI 17.7–64.5%). The TgsGp-qPCR specificity was 100% (6/6, 95% CI 61.0-100.0%).

### Table 4

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>% Sensitivity (n/N, 95% CI)</th>
<th>% Specificity (n/N, 95% CI)</th>
<th>% PPV (n/N, 95% CI)</th>
<th>% NPV (n/N, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypanolysis (in parallel)</td>
<td>85.3 (29/34, 68.9–95.0)</td>
<td>92.9 (39/42*, 80.5–98.5)</td>
<td>90.6 (29/32, 75.0–98.0)</td>
<td>88.6 (39/44, 75.4–96.2)</td>
</tr>
<tr>
<td>TL LiTat 1.3</td>
<td>79.4 (27/34, 62.1–91.3)</td>
<td>92.9 (39/42*, 80.5–98.5)</td>
<td>90.0 (27/30, 73.5–97.9)</td>
<td>84.8 (39/46, 71.1–93.7)</td>
</tr>
<tr>
<td>TL LiTat 1.5</td>
<td>55.9 (19/34, 37.9–72.8)</td>
<td>92.9 (39/42*, 80.5–98.5)</td>
<td>86.4 (19/22, 65.1–97.1)</td>
<td>72.2 (39/54, 58.4–83.8)</td>
</tr>
<tr>
<td>ELISA/ T.b. gambiense</td>
<td>67.6 (23/34, 49.5–82.6)</td>
<td>95.3 (41/43, 84.2–99.4)</td>
<td>92.0 (23/25, 74.0–99.0)</td>
<td>78.8 (41/52, 65.3–88.9)</td>
</tr>
<tr>
<td>m18S qPCR</td>
<td>44.1 (15/34, 27.2–62.1)</td>
<td>97.7 (42/43, 87.7–99.9)</td>
<td>93.8 (15/16, 69.8–99.8)</td>
<td>68.9 (42/61, 55.7–80.1)</td>
</tr>
<tr>
<td>LAMP</td>
<td>36.4 (12/33*, 20.4–54.9)</td>
<td>93.0 (40/43, 80.9–98.5)</td>
<td>80.0 (12/15, 51.9–95.7)</td>
<td>65.6 (40/61, 52.3–77.3)</td>
</tr>
</tbody>
</table>

### Discussion

The HAT prevalence observed among the study participants during the 3 years of passive screening was 2.0%. The overall HAT prevalence reported by the national program in passive screening in the same prefectures in 2017 and 2018 was respectively 0.98 and 0.39% [3]. The DiTECT-HAT-WP2 study was set up in a small selection of experienced reference hospitals and health posts, which probably explains the
difference. The relatively high prevalence allowed to successfully assess the sensitivity, specificity, positive and negative predictive value of clinical symptoms and signs, HAT rapid diagnostic tests and laboratory tests for diagnosis of HAT.

Although there may be geographical and stage specific variations, the clinical picture of HAT has been described in detail [9, 10]. However, within a context of passive screening for HAT, it is important to consider the frequency of signs and symptoms in non-HAT affected individuals visiting the health infrastructure as well as for proposing criteria for HAT referral. This was well illustrated in the actual study by the criterion “presence of recurrent fever not responding to anti-malarial medication”. Fever is considered among the leading symptoms of HAT [26, 27] and has previously been reported at 97% and 73.4% frequency in Guinean HAT patients [28, 29]. It was also one of the most frequent symptoms (89.6%) in our HAT patients but in univariable analysis, fever was negatively associated with HAT and the odds that participants with fever would have HAT were below one, suggesting fever would be protective. This is probably an artefact given that it was common also in non-HAT affected participants (96.1%). Indeed, in the multivariable analysis, both fever and having a headache for a long duration (the 2 clinical signs most frequently observed in the in the study participants) were not statistically significant. Among the 13 clinical symptoms and signs serving as inclusion criteria, using multivariable logistic regression, we were able to identify 4 key clinical presentations which could be used to select, among a health care seeking population, individuals at increased risk for HAT and which should be referred for further HAT screening. The presence of either enlarged lymph nodes, and/or itching, and/or weight loss and/or motor disorders was in our study 97.9% sensitive for HAT and had 75.6% specificity, resulting in a PPV of 7.7%. A combination of at least 2 signs or symptoms increased the PPV to 39.3%, but resulted in a decrease in sensitivity. In particular presence of enlarged lymph nodes has previously been identified in 90% and 93% of HAT patients in Guinea, while occurrence of itching has been reported with frequencies of 93% and 29.4% [28, 29]. In the present study, enlarged lymph nodes and itching were present in respectively 77.1% and 50.0% of HAT patients, and it should be underlined that itching was retained as an inclusion criterion in the study at the specific request of the Guinean national HAT program. Previous independent studies on clinical presentation-based HAT diagnostic referral [11] identified sleep problems, neurological problems and weight loss as core symptoms in South Sudan, with or without oedema, swollen lymph nodes or proximity to livestock. Their diagnostic algorithms, based on these clinical presentations, had sensitivities up to 92.6% and NPVs and PPVs of maximum 8.7% [11]. Although itching was also significantly associated with HAT in South Sudan, it was not retained in the algorithms. In the Republic of Congo, enlarged lymph nodes, oedema, fever, headaches and itching were considered for establishing a clinical presentation based diagnostic algorithm for identifying HAT [30]. In Côte d'Ivoire, odds for having a positive RDT for HAT were increased in study participants with sleep disturbances, motor disorders, convulsions, important weight loss and psychiatric problems. In the Ivorian part of the DiTECT-HAT-WP2 study, only 2 HAT patients were identified, and the overall frequency of enlarged lymph nodes and itching was low (3.6 and 8.2% respectively). Finally, our finding in the present study that males were at higher odds to have HAT than females, is in line with previous observations in Guinea, and has been linked to activities like rice growing, salt extraction, fishing and wood collection, which expose men more to the
vector [29]. The hypothesis of unequal access to the health system [29], disfavouring women, can probably be excluded, as slightly more women were included in the present study. On the other hand, men traditionally participate less to active screening [29], which could explain why, once they start developing second stage HAT symptoms, are more easily picked up through passive screening.

The combined seroprevalence during this study was 5.2%, ranging from 1.9 to 4.7% for the individual RDTs. As for the HAT prevalence, this was again higher than the overall seroprevalence of 1.72 and 0.98 previously reported in 2017 and 2018 [3]. The specificities of the 3 RDTs observed in Guinea confirm those reported in Côte d’Ivoire [21], and in other prospective evaluations in Central Africa [12, 13, 31, 32]. The PPV of the 3 RDTs, ranging between 45.2 and 66.7% are however similar to those observed in passive case detection in Guinea for 2017–2018 [3]. Sensitivity of HAT Sero-K-Set was 100%, confirming the high sensitivity for this test in prospective studies in the Democratic Republic of the Congo [12, 32]. For SD Bioline HAT, a wide variation of sensitivities has been reported from different prospective studies in Central Africa, ranging from 59.0% [31] over 89.3% [13] to 92.0% [33]. In a retrospective study on stored plasma originating mainly from Guinean HAT patients, a sensitivity of 99.6% was observed [19], although this might have been an overestimation due to selection bias using CATT/ T. b. gambiense. In the present study, the 93.8% sensitivity of SD Bioline HAT was close to the higher sensitivity values reported for the Democratic Republic of the Congo [33]. The sensitivity of 59.6% observed with rHAT Sero-Strip in the present study was low compared to the >97.5% sensitivities obtained using stored specimens in a laboratory evaluation [34]. This was the first evaluation of the sensitivity of the rHAT Sero-Strip dipstick test under field conditions and it cannot be excluded that transport stress, the higher environmental temperatures or the humidity might have affected test stability. In parallel or in series combination of tests, with or without rHAT Sero-Strip did not improve diagnostic performance, probably because of the agreement between the HAT Sero-K-Set and SD Bioline HAT test results.

Evaluation of the diagnostic performance of the parasitological tests was not an objective of our study and not all tests were systematically performed on all RDT positives, but our results confirm the relatively high sensitivity of lymph examination in Guinea [8, 29]. Indeed, 26/48 (54.2%) of the HAT patients had trypanosomes upon microscopic examination of the lymph node exudate.

Unfortunately, DBS were missing for a relatively high number of RDT positives. The specificity of the 4 laboratory tests in this study was similar as for passive screening in Côte d’Ivoire [21]. For ELISA/ T. b. gambiense and trypanolysis, specificity was lower than in active screening in Burkina Faso [35]. However, among the 6 DBS positives considered as non-HAT for determination of the diagnostic performance (Figs. 1 and 4), two participants died before they could be re-examined. They had respectively 2/4 and 3/4 key symptoms and signs, were positive in 3/3 and 2/3 RDTs and were both positive in trypanolysis and ELISA/ T. b. gambiense but not in the molecular tests. It cannot be confirmed nor excluded that these were real HAT patients, thus the specificity values of trypanolysis and ELISA/ T. b. gambiense might have been underestimated. The sensitivities of trypanolysis and ELISA/ T. b. gambiense observed in the present study were modest. It has previously been demonstrated that the sensitivity of trypanolysis and inhibition ELISA is lower on DBS compared to plasma [14, 36]. In DR Congo, trypanolysis on DBS was 95.1%
sensitive [37], while ELISA/ *T. b. gambiense* was estimated to be 82.2% sensitive [38]. The low sensitivities for the molecular tests LAMP and m18S qPCR were not a complete surprise. Firstly, DBS have been shown to be suboptimal for PCR and better results are obtained with nucleic acid preservation in different types of stabilisation buffers [37, 39]. Secondly, LAMP and m18S qPCR on DBS have been shown to have limited analytical sensitivity (100 and 1000 trypanosomes/ml respectively)[18], which is lower than that of mAECT-BC (10 trypanosomes/ml)[8], which was used together with lymph and CSF examination, to diagnose HAT in the present study. Finally, a prolonged or suboptimal storage of DBS could also have affected the sensitivity of both the serological and the molecular laboratory tests: DBS were not systematically shipped to the reference laboratory, and exposure to humidity, despite storage with silica gel, cannot be entirely excluded.

Some limitations of this multi-country study have already been discussed in detail elsewhere [21], including non-inclusion of individuals without symptoms, incomplete inclusion of individuals presenting with symptoms or signs at the CDT or SSS, the assumption that individuals testing negative in all 3 RDTs are not affected by HAT without carrying out parasitological examinations, imperfect sensitivity of parasitological techniques used as a gold standard. A number of additional limitations apply to the present study in Guinea. All the 48 HAT patients that were diagnosed suffered from stage 2 HAT. This is a known problem in passive screening [3], not only in Guinea [11, 26, 27, 40]. As a result, the proposed key clinical presentation might have high sensitivity for stage 2 HAT, but its real ability to pick up stage 1 HAT patients, which may be asymptomatic or have only mild symptoms, remains to be determined. Furthermore, itching and enlarged lymph nodes in particular are relatively well known as clinical symptoms and signs of HAT in Guinea. It is possible that clinicians responsible for inclusion gave more attention to these clinical presentations compared to others, which could have remained under-detected. Many DBS of RDT positives were missing, leading to relatively large confidence intervals in diagnostic test performance estimations. Moreover, the delay between collection and analysis of DBS might have affected sensitivity of the tests. Finally, newer second generation RDTs, in particular SD Bioline HAT 2.0 with recombinant antigens were not available during the study, despite its large scale evaluation in the Democratic Republic of the Congo prior to the start of the present study [31]. Production of rHAT Sero-Strip has since been discontinued, while SD Bioline HAT with native antigens is nowadays unavailable.

The actual study also has important strengths. The high HAT prevalence in Guinea allowed us to assess the association between clinical symptoms and signs and HAT in Guinea and the diagnostic performance of the combination of 4 key clinical presentations, 3 RDTs, and consequent laboratory tests on DBS of RDT positives. A similar study in Côte d’Ivoire [21] allowed us to associate the clinical picture with RDT positivity and to assess test specificity of RDTs and laboratory tests, but included only 2 HAT patients. The Guinean HAT control program paid particular attention to actively retrieving RDT positives, resulting in a limited loss to follow-up.

**Conclusions**
Our findings might have several practical applications. First, taking into account that active screening becomes less cost effective, integration of passive screening in the existing health system is gaining importance. In this context, the number of health facilities able to diagnose HAT has considerably increased, in Guinea and other HAT endemic countries [1]. In passive case detection, referral to HAT RDT screening is based on clinical examination of the presenting population. Based on our results, we can propose to Guinean health workers and clinicians a relatively simple set of criteria with high sensitivity for selecting individuals to be further tested using HAT RDTs. Referral based on presence of at least one of these 4 key clinical criteria, would result in a reduction of almost 70% of the HAT RDTs that would need to be carried out, which in turn would reduce by 20% the number of RDT positives that would need to be further tested with parasitological examinations. This would diminish the burden on the health system, as well as for the RDT positive individuals, who sometimes travel long distances from their home to the district confirmation laboratory. Performance of both HAT Sero-K-Set and SD Bioline HAT is sufficient for referring RDT positives for microscopic examination. Taking into account the high probability that non-toxic oral drugs to treat both stages of HAT will be available in the near future, a target product profile has been established by the World Health Organization for a gambiense HAT test to identify individuals with suspected but microscopically unconfirmed g-HAT infection, eligible for treatment with safe and easy-to-use medicines [41]. Both RDTs largely meet the minimum requirements for specificity, while HAT Sero-K-Set meets the desirable sensitivity and SD Bioline HAT approaches the minimal sensitivity. Awaiting approval and implementation of such new drugs, microscopy remains the gold standard. However, taking into account the imperfect sensitivity of microscopy, DBS testing may help to discriminate individuals testing false positive in RDTs from true HAT patients which need to be re-examined for confirmation of HAT. All laboratory tests on DBS showed sufficient specificity, but only the antibody detection tests, and in particular trypanolysis had sufficient sensitivity. More care should however be given to correct collection, storage and shipment of DBS, and to minimising the delay between collection and testing of the specimens. The diagnostic performance, in particular sensitivity of the molecular tests carried out on DBS, was insufficient although blood collection methods might have been sub-optimal.

In the future, priority should be given to assessing the diagnostic performance of new generation RDTs. The diagnostic performance of new laboratory tests should also be assessed. For example, inhibition ELISA could replace trypanolysis [36], increasing the feasibility of implementation of DBS testing in-country and reducing DBS storage time. New nucleic acid detection tests including SHERLOCK [42] or (RT)-qPCRs [39] should be evaluated prospectively, including optimized specimen collection methods. Furthermore, the decision for the most suitable diagnostic algorithm for passive HAT case detection in Guinea should also be guided by cost-effectiveness analysis.

Abbreviations

CDT
Centre for diagnosis and treatment
CSF
Cerebrospinal fluid
Declarations

Ethics approval and consent to participate

The study in Guinea was part of the multi-country diagnostic clinical trial “Diagnostic tools for human African trypanosomiasis elimination and clinical trials work package 2, passive case detection” (DiTECT-HAT-WP2), registered on ClinicalTrials.Gov under identifier NCT03356665. Before initiation of the study, DiTECT-HAT-WP2 received ethical clearance from the Advisory Committee on Deontology and Ethics of the French National Institute for Research on Sustainable Development (plenary meeting of 17-20 October 2016), of the Institutional Review Board of the Institute of Tropical Medicine in Antwerp Belgium (reference 1133/16), and of the Ethics Committee of the University of Antwerp (Belgian registration number B300201730927). In Guinea, DiTECT-HAT-WP2 was approved by the Comité National d’Ethique pour la Recherche en Santé (CNERS, reference 025/CNERS/17). Potential study participants were informed how and why the study was carried out, and gave their written informed consent before inclusion in the study. For minor participants, an assent was obtained and written informed consent was provided by the parents or legal guardians. All clinical investigations were conducted according to the Declaration of Helsinki.
Consent for publication

Not applicable

Availability of data and materials

The public sharing of personal health data is subject to the General Data Protection Regulation. The health data underlying the findings described in the manuscript can therefore not be made public. Metadata are available via Lejon V, Camara O, Camara M; Ilboudo H, Kaboré J, Compaoré CFA, Buscher P, Bucheton B, 2022, "Passive case detection of Human African Trypanosomiasis in Guinea: symptoms and signs, rapid diagnostic test results and laboratory test results", DataSuds, https://doi.org/10.23708/ZDD00W [43]. The datasets generated and analysed in the present manuscript will be made available to qualified researchers upon request and after signing a confidentiality agreement. Data requests may be sent to the Institut de Recherche pour le Développement (IRD) data administrator (data@ird.fr).

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

All authors participated in writing, reviewing and editing of the draft, and approved the final manuscript. VL was responsible for conceptualization, formal Analysis, data curation, funding acquisition, methodology, project administration and original draft preparation. OC participated in investigation and methodology. MC was involved in conceptualization, methodology, project administration, and supervision. LCF performed formal analysis and validation. HI was involved in conceptualization and investigation. JK participated to investigation and supervision. CFAC performed investigations. EMF participated in conceptualization and methodology. PB was involved in conceptualization, data curation, funding acquisition, and methodology. BB participated in conceptualization, formal Analysis, methodology, and project administration.

Acknowledgements

We acknowledge staff members of the Guinean HAT National Elimination Program, and the HAT team of the Centre International de Recherche-Développement sur l’Elevage en Zone Subhumide in Bobo-
Dioulasso (Burkina Faso). We also acknowledge the health staff members from the health facilities involved in the study.

References


**Figures**
**Figure 1**

**DITECT-HAT-WP2 study conduct and test results in Guinea.** RDT rapid diagnostic test. mAECT-BC mini anion exchange centrifugation on buffy coat. DBS dried blood spot. *HAT confirmed by CSF examination. ND: not done. HATÅ: HAT positive. HATÆ: HAT free.
Figure 2

**Frequency of 13 clinical symptoms and signs in HAT and non-HAT affected study participants.** The figure contains data for 48 HAT and 2297 non-HAT participants with the exception of * only 19 HAT and 1294 non-HAT female participants.
Individual RDT results of HAT patients and HAT free participants. The Venn diagram shows results in the RDTs HAT Sero-K-Set, rHAT Sero-Strip and SD Bioline HAT of 48 HAT patients and 2297 HAT free participants. $K^*$ HAT Sero-K-Set not performed, $S^*$ rHAT Sero-Strip not performed, $B^*$ SD Bioline HAT not performed, HAT$\hat{\text{A}}$: HAT patients, HAT$\hat{\text{Æ}}$: HAT free participants.
**Figure 4**

**Laboratory test results on dried blood spots.** The Venn diagram shows the results of trypanolysis, ELISA/ *T.b. gambiense*, LAMP and m18S qPCR on DBS from 34 HAT patients (HATÅ) and 43 HAT negatives (HATÆ) who all tested rapid diagnostic test positive. $T^+$ trypanolysis not performed, $L^+$ LAMP not performed, U died after the first parasitological examination at inclusion.