Diagnostic Accuracy and Feasibility of Serological Tests on Filter Paper Samples for Outbreak Detection of *T.b. gambiense* Human African Trypanosomiasis

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Abstract. Control of human African trypanosomiasis (HAT) in the Democratic Republic of Congo is based on mass population screening by mobile teams; a costly and labor-intensive approach. We hypothesized that blood samples collected on filter paper by village health workers and processed in a central laboratory might be a cost-effective alternative. We estimated sensitivity and specificity of micro-card agglutination test for trypanosomiasis (micro-CATT) and enzyme-linked immunosorbent assay (ELISA)/*T.b. gambiense* on filter paper samples compared with parasitology-based case classification and used the results in a Monte Carlo simulation of a lot quality assurance sampling (LQAS) approach. Micro-CATT and ELISA/*T.b. gambiense* showed acceptable sensitivity (92.7% [95% CI 87.4–98.0%] and 82.2% [95% CI 75.3–90.4%]) and very high specificity (99.4% [95% CI 99.0–99.9%] and 99.8% [95% CI 99.5–100%]), respectively. Conditional on high sample size per lot ($\geq 60\%$), both tests could reliably distinguish a 2% from a zero prevalence at village level. Alternatively, these tests could be used to identify individual HAT suspects for subsequent confirmation.

INTRODUCTION

West African human African trypanosomiasis (HAT) is a slowly progressing chronic disease, which is usually fatal if left untreated. In the initial stages of the disease symptoms are usually absent or non-specific.¹ By the time patients consult a health professional the disease is often advanced and has spread to the central nervous system. As a result, the patient may require more toxic and expensive treatment. Moreover, the community has been at risk because the patient has been a potential source of infection for the tsetse fly vector for a prolonged period.² Therefore, the current HAT control strategy in the Democratic Republic of the Congo (DRC) is based on active screening of the population at risk, with the aim to find and treat cases as early as possible. Such screening is carried out by mobile teams that spend several days in each village. On the spot, they screen the population by a card agglutination test for trypanosomiasis (CATT), followed by parasitological confirmation tests for those who are CATT positive.^{3,4} The total population at risk for HAT in the DRC is estimated at 12.6 million; the reported annual incidence peaked at over 25,000 cases in 1997 and was reduced to just over 8,000 cases by 2006.⁵ Thirty-five mobile teams are currently active, each able to screen a maximum of 60,000 persons per year. Lutumba and others⁶ estimated that in each screening round about 40% of existing cases are missed, mainly because of suboptimal participation by the target population and inadequate sensitivity of confirmatory tests.⁴ Ninety-eight percent of HAT control activities in the DRC is funded by international development aid.⁷

If HAT control measures are successful, prevalence levels may be reduced to below 0.1%. At such low prevalence rates, continuation of active case finding becomes a problem.⁴ The population no longer perceives HAT as a threat and participation rates drop; governments and donors start questioning the rationale behind maintaining costly programs that detect so few cases. Integration of HAT control activities into the gen-

eral health care system is a logical next step.⁵ However, such integration faces many challenges, mainly because of the lack of a sensitive and specific diagnostic test and the lack of a treatment that is easy to administer and safe. On top of this there is the problem of the unspecific clinical picture in early stages and the diagnosis is therefore often missed by health facilities; as was described for East African HAT by Odiit and others⁸ in Uganda. If screening activities are abandoned, the disease usually reemerges within a time span that can vary from 3 to 50 years.^{9–11} Therefore, some form of continuous surveillance is needed in low endemic areas.

A perfect surveillance system of low prevalence areas should be sensitive to detect all outbreaks at an early stage, but at the same time specific, to avoid raising the alarm without necessity. Laveissière and others¹² explored the idea of using samples collected on filter paper as a surveillance tool in Côte d'Ivoire. Higher population coverage was achieved by general health workers collecting samples on filter paper over a 2-month period than by a mobile team spending 10 days in the same region; the latter approach being five times more costly.

Whereas Laveissière and others used results of serological tests on filter paper samples to identify individual suspect cases, such approach could, in theory, also be used to identify “suspect villages” in analogy with the system of lot quality assurance sampling (LQAS) used to monitor vaccination coverage and other healthcare programs.^{13,14} In LQAS, the population is regrouped into lots and a random sample is then taken from each lot. The lot is rejected if the number of defects observed in the sample exceeds a maximum specified limit (or “threshold”). In our case, a village would be a lot and the number of defects in the sample would equate the number of positive results on HAT serological tests. Any LQAS scheme has to identify the sample size to be tested per lot and the “threshold” value within that sample that leads to rejection of the whole lot. For HAT surveillance, the scheme should take into account the probability of false positive serological results, which are more frequent at low prevalence of disease.

Different tests can be performed on blood samples collected on filter paper, such as micro-CATT, LATEX/*T.b. gambiense* and enzyme-linked immunosorbent assay (ELISA)/*T.b. gambiense*. Micro-CATT is a serological test that uses the

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same reagent as the CATT test.¹⁵ Several studies, although using different test protocols, report on validation of micro-CATT under field conditions.^{11,14,16–18} Specificity estimates range from 93.7% to 100%; sensitivity was estimated to be 94% by Chappuis and others¹⁸ and 94.2% by Noireau and others.¹⁷ The LATEX/*T.b. gambiense* is a rapid LATEX agglutination test for detection of antibodies in patients infected with *Trypanosoma brucei gambiense*. The reagent consists of beads coated with a mixture of three variable surface antigens of bloodstream form trypanosomes.¹⁹ Penchenier and others²⁰ evaluated LATEX/*T.b. gambiense* as a tool for mass screening in Cameroon and the Central African Republic. Sensitivity was 100%, whereas specificity ranged from 96.1% in the Central African Republic to 97.6% in Cameroon. The ELISA/*T.b. gambiense* is an antibody-detection test based on reaction of specific antibodies with purified variable surface glycoproteins from *T.b. gambiense* fixed in an ELISA plate.²¹ During a field evaluation in Sudan, Elrayah and others²² found specificities of 98.5% for ELISA/*T.b. gambiense* on plasma and on eluates from filter paper.

The objectives of this study were 1) to compare the diagnostic accuracy of micro-CATT, LATEX/*T.b. gambiense*, and ELISA/*T.b. gambiense* on capillary blood samples collected on filter paper under field conditions (by mobile teams); and 2) to assess the potential of a LQAS-based surveillance system for outbreak detection of West African HAT in low prevalence areas.

MATERIALS AND METHODS

Study population. The study population lives in two HAT-endemic regions in the DRC, East Kasai, and Bas-Congo, which were selected on the basis of different HAT prevalence levels. The HAT prevalence in East Kasai is estimated to be above 1%, in Bas Congo it is estimated at < 0.03% (Kande V, personal communication). For practical and ethical reasons, study subjects were recruited among the beneficiaries of the screening program of the national HAT control program. All those participating in the routine active case finding sessions by the mobile team were eligible, conditional on written informed consent from the study subject or his/her guardian. There were no age restrictions.

Sample size for estimation of diagnostic accuracy. Assuming a specificity of 98%, a sample size of 1,600 true negatives is required to estimate specificity at a precision of 0.5%. Based on an expected sensitivity of micro-CATT, ELISA/*T.b. gambiense*, and LATEX/*T.b. gambiense* of 90% and a desired precision of 10%, we needed to recruit a minimum 35 true cases of HAT (i.e., confirmed with positive parasitology).

Data collection. Mobile teams performed active case finding using the routine diagnostic protocols as prescribed by the national program. The CATT on whole blood was used as a screening test; those testing positive were subjected to a series of confirmation tests, which includes lymph node puncture, thick film examination, capillary tube centrifugation test (CTC), and miniature anion exchange column test (mAECT).^{23,24} Tests were performed sequentially and once parasitological confirmation was obtained no further confirmation tests were done. Any parasitologically confirmed patient was subjected to a lumbar puncture to determine the stage of the disease. In addition to those routine procedures, blood samples were taken on Whatman no. 4 filter paper, left to dry, and stored in a sealed plastic bag with silica gel at ambient temperature. Each sample

was given a serial number and stored anonymously. The CATT whole blood and parasitology results were recorded under the same code number in a register. Plastic bags with filter paper samples were sent to the Institut National de Recherche Biomédicale (INRB) laboratory in Kinshasa by road or air transport. The time between blood collection and testing varied from a minimum of 1 week to a maximum of 2 months.

Laboratory procedures. The filter papers were cut into pieces at INRB and were further processed at the INRB and at the Institute of Tropical Medicine (ITMA) in Antwerp; both laboratories performed micro-CATT, LATEX/*T.b. gambiense*, and ELISA/*T.b. gambiense*.

Micro-CATT was performed as described in Chappuis and others.¹⁸ The LATEX/*T.b. gambiense* was performed by punching out one disc of 6 mm diameter of the dried blood spot, and eluting it in a microplate with 60 µL of buffer for 1 hour with gentle shaking. A vial of LATEX/*T.b. gambiense* was reconstituted with 1 mL of buffer. Twenty µL of the filter paper eluate and 20 µL of the LATEX/*T.b. gambiense* reagent were spread out on the reaction zone of the agglutination card and rocked on a rotator (ITMAS type B2) for 5 minutes at 70 rpm. Agglutination patterns were scored as negative or positive.

The ELISA/*T.b. gambiense* was performed as described earlier by Lejon and others²⁵ with small modifications. Two discs of 5 mm diameter were punched out of the dried blood spot and left overnight in 1 mL of PBS-Blotto (0.01 M phosphate, pH 7.15, 0.2 M NaCl, 0.05% w/v NaN₃, 1% w/v skimmed milk powder; Fluka, Buchs, Switzerland), containing 0.05% v/v Tween 20 (Merck, Schuchardt, Germany). Microplates (Maxisorp, Nunc, Roskilde, Denmark) were coated overnight with 150 µL/well of a mixture of purified variable surface glycoproteins of *T.b. gambiense* LiTat 1.3, LiTat 1.5, and LiTat 1.6 each at a concentration of 0.66 µg/mL. Antigen-free control wells were left empty. Plates were blocked for 1 hour at ambient temperature, with 350 µL/well of PBS-Blotto (0.01 M phosphate, pH 7.15, 0.2 M NaCl, 0.05% w/v NaN₃, 1% w/v skimmed milk powder; Fluka). For testing, antigen containing and antigen-free wells were filled with 150 µL of eluate (in duplicate). A strongly positive control serum, diluted 1:150 in PBS-Blotto, was tested in quadruple in each plate. The plate was incubated for 1 hour and washed 3 times with 350 µL/well of PBS-Tween (0.01 M phosphate, 0.14 M NaCl, 0.05% v/v Tween 20, pH 7.4). Goat anti-human IgG (H+L) peroxidase (Jackson Immuno Research, West Grove, PA) was diluted in PBS-Tween to a final concentration of 1:40,000, and incubated for 30 minutes (150 µL/well). After 5 washes, wells were incubated for 1 hour with 150 µL ABTS substrate-chromogen solution. The latter was prepared from 50 mg ABTS (2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid; Boehringer, Mannheim, Germany) dissolved in 100 mL of ABTS-buffer (phosphate-citrate-sodium perborate solution, pH 4.6; Boehringer). The optical density (OD) was read at 415 nm (Multiskan RC version 6.0, Labsystems, Helsinki, Finland). The OD of the antigen-free control well was subtracted from the OD of the corresponding antigen containing well, the average was taken, and the result expressed as the percent positivity of the positive control serum included in the plate.

Statistical analysis. For data analysis and simulations, we used Stata/IC version 10.1 (Stata Corp., College Station, TX) and R 2.10.0 (The R Project for Statistical Computing, Vienna, Austria). We determined sensitivity and specificity of individual diagnostic tests using 2 × 2 contingency tables and

inter-rater agreement using the Kappa-index.²⁶ Persons with a history of previous treatment were excluded; parasitological confirmation according to the national algorithm was used as reference standard.⁴ Specificity of each test was calculated based on the results of the low prevalence area (Bas Congo); sensitivity was calculated based on results from the high prevalence area (East Kasai). For calculating the Kappa-index we combined results from both areas. Receiver–Operator–Characteristics curves were constructed to determine the optimal cutoff points for ELISA/*T.b. gambiense* (i.e., the cutoff resulting in the highest Youden-index).²⁷

In a second step, we used Monte Carlo simulation to assess the performance of a (hypothetical) early warning system at village level based on an LQAS approach. We assumed villages (lots) would have a similar size of $N = 1,000$ and identified the sample size per lot and the number of positive samples required (= threshold) to declare a village as affected by an outbreak (= HAT prevalence equal or above 2%). Thresholds were calculated for different sample sizes varying between 400 and 900 (i.e., 40–90% of the hypothetical village population). For each threshold obtained, we simulated how often the alarm would be raised at various HAT prevalence levels. The probability of not reaching the alarm threshold and thus not raising the alarm in case the actual prevalence is equal to or above 2% was fixed at 5% or less.

Ethical clearance. The study was approved by the ethics committees of the University of Antwerp (Belgium) and of the National Human African Trypanosomiasis Control Program (PNLTHA) in Kinshasa (DRC).

RESULTS

Study population characteristics. Excluding persons with a history of previous treatment, a population of 3,212 persons was screened in two provinces, 1,507 in Bas Congo and 1,705 in East Kasai. Out of those, 262 (8.2%) tested positive by CATT on whole blood; 4 (0.3%) in Bas Congo and 258 (15.1%) in East Kasai. Among those 262 CATT positives, 100 parasitologically confirmed cases were identified, all residents of East Kasai (5.9% of the population screened in East Kasai).

Sixty-seven cases had a positive lymph node aspirate, 2 had a positive thick film, 44 had a positive CTC, and 3 had a positive mAECT. In 21 cases, parasites were found in the cerebrospinal fluid.

Results of screening tests. Unexpectedly, LATEX/*T.b. gambiense* showed very poor sensitivity both at INRB (16.0%, 95% confidence interval [CI] 8.7–23.3%) and at ITMA (42.3%, 95% CI 32.2–52.3%). We subsequently tested the batch used against

standard reference sera and observed a 4-fold reduction in end-titer when compared with initial results obtained immediately after production in the quality assurance scheme. Apparently, the batch had lost much of its reactivity; results with this test were therefore discarded from further analysis and will not be further reported on.

For the other tests, sensitivity and specificity (based on the results from the low prevalence area only) varied between the different tests and between the different laboratories; for ELISA/*T.b. gambiense* the results also vary according to the cutoff points chosen (Table 1).

At INRB, 89 out of 96 samples from confirmed cases tested positive in Micro-CATT; corresponding to a sensitivity of 92.7% (95% CI 87.4–98.0%). No samples from cases were tested at ITMA because in most cases the quantity of blood collected was insufficient.

Out of 1,037 non-case samples tested at INRB, six turned out positive; equivalent to a specificity of 99.4% (95% CI 99.0–99.9%). At ITMA, all 953 samples of non-cases tested turned out negative; equivalent to a specificity of 100% (95% CI 99.5–100%). Inter-rater agreement could not be assessed as there were no positive samples at ITMA.

For ELISA/*T.b. gambiense*, we first determined the optimal cutoff point based on receiver operating characteristic (ROC) curves for the high prevalence area, East Kasai. Areas under the curve of 0.965 (95% CI 0.939–0.990) and 0.972 (95% CI 0.953–0.991) were obtained at INRB and ITMA, respectively (Figure 1). Sensitivity and specificity vary according to the cutoff point chosen. At INRB, a cutoff point of 18% for the optical density of the positive control resulted in the highest Youden-index with 95.0% sensitivity (95% CI 90.6–99.3%) and 95.2% specificity (95% CI 94.1–96.2%). Increasing the cutoff point to 32% resulted in a sensitivity of 82.8% (95% CI 75.3–90.4%) and a specificity of 97.1% (95% CI 96.3–98.0%). At ITMA, the optimal cutoff point was 52%, resulting in a sensitivity of 96.9% (95% CI 93.4–100%) and a specificity of 96.1% (95% CI 95.1–97.1%); specificity could be increased to 97.0% (95% CI 96.1–97.8%) at the cost of decreasing sensitivity to 91.8% (95% CI 86.2–97.3%) if 65% was chosen as a cutoff point.

Applying these cutoff points to the low prevalence area resulted in a specificities of 96.5% (95% CI 95.3–97.8%) for the lower cutoff point (18%) and 99.8% (95% CI 99.4–100%) for the higher cutoff point (32%) at INRB. At ITMA, specificity was 99.6% (95% CI 99.2–99.9%) with the 52% cutoff point and 99.8% (95% CI 99.5–100%) when using 65% as a cut off.

Combining the results for the two areas and comparing results of ITMA at a cutoff point of 52% with those of INRB at a cutoff point of 18%, a kappa of 0.84 was obtained.

TABLE 1
Sensitivity, specificity, and inter-rater agreement (Kappa) for 3 test formats for HAT on capillary blood (filter paper)*

Test laboratory	Sensitivity (95% confidence interval)		Specificity (95% confidence interval)		Inter-rater agreement (Kappa)
	ITMA	INRB	ITMA	INRB	
MicroCATT	NA	92.7% (87.4–98.0%)	100% (99.5–100%)	99.4% (99.0–99.9%)	NA
ELISA/ <i>T.b. gambiense</i>					
Low cutoff† (52% at ITMA, 18% at INRB)	96.9% (93.4–100%)	95.0% (90.6–99.3%)	99.6% (99.2–99.9%)	96.5% (95.3–97.8%)	0.84
High cutoff† (65% at ITMA, 32% at INRB)	91.8% (86.2–97.3%)	82.8% (75.3–90.4%)	99.8% (99.5–100%)	99.8% (99.4–100%)	0.88

* ITMA = Institute of Tropical Medicine; INRB = Institut National de Recherche Biomédicale; Micro-CATT = micro-card agglutination test for trypanosomiasis; NA = not available; ELISA = enzyme-linked immunosorbent assay.

† Expressed as percentage of the optical density of a positive control.

FIGURE 1. ROC curves for ELISA/*T.b.gambiense* based on results of INRB, Kinshasa (left side) and ITMA, Antwerp (right side) for the high prevalence area.

For the higher cutoff points (65% at ITMA versus 32% at INRB), kappa was 0.88.

Simulation of LQAS approach to detect HAT outbreaks.

Assuming an average village population size of 1,000 and accepting a maximum HAT prevalence of 2% for rejecting a lot (i.e., raising the alarm that the village should be screened by a mobile team), we calculated the alarm thresholds for different sample sizes per lot (see Table 2). We found that conditional on high sample sizes ($\geq 60\%$ of the lot) the system can reliably distinguish zero prevalence from 2% prevalence. For both micro-CATT and ELISA, chances of surpassing these thresholds under conditions of zero prevalence were less than 0.5% provided the sample size was at least 60% (Table 2).

DISCUSSION

We validated two different serologic test formats that can be used on samples collected on filter paper, based on a sample of 100 true HAT cases and 1,507 non-cases. Micro-CATT and ELISA/*T.b. gambiense* both came out as promising candidates for a surveillance system in low prevalence settings because of a combination of a fairly high sensitivity and a very high specificity.

Micro-CATT sensitivity and specificity at INRB were 92.7% and 99.4%, respectively; at ITMA specificity was 100% but sensitivity could not be assessed because no samples from the high prevalence area were tested. Disadvantages of the technique are mainly related to the number of confettis required, nine per sample. A sufficiently large surface of blood impregnated filter paper is needed to perform the test correctly. Furthermore, because of the need of cutting out these confettis, the test is relatively labor intensive. MicroCATT has the advantage of not requiring any equipment other than the standard kit for the CATT test plus some micro-titer plates. When used in a low income setting, the disadvantage of having to cut nine confettis per sample is easily overcome because this does not require a skilled worker.

ELISA/*T.b. gambiense* performed very well both at the INRB laboratory in the DRC and at ITMA in Belgium. This is reflected in areas under the ROC curve of 96.5% and 97.2%, respectively, which is considered excellent.²⁸ At the higher cutoff point specificity in the DRC was 99.8%, with a still acceptable sensitivity of 82.8%. Interrater agreement was good with kappa values of 0.84 to 0.88 depending on the cutoff point chosen. In its current format the test is based on three different variable surface glycoproteins; omitting one antigen is still an

TABLE 2

Probability of the alarm threshold being surpassed for various true HAT prevalence rates and depending on sample size in LQAS for micro-CATT and ELISA/*T.b. gambiense* for an average lot size of $N = 1,000^*$

Test	Specificity	Sensitivity	Number sampled (%)	Threshold (proportion of positive tests)	Probability of alarm threshold being surpassed at HAT prevalence levels of:				
					0%	0.5%	1%	1.5%	2%
Micro-CATT	99.4%	92.7%	400 (40%)	6(1.5%)	3.5	24.6	58.1	84.5%	95.3%
			500 (50%)	8(1.6%)	1.2	14.7	50.5	82.2%	95.6%
			600 (60%)	10(1.7%)	0.4	8.1	42.5	81.4	96.9
			700 (70%)	12(1.7%)	0.1	4.7	35.9	81.1	97.3
			800 (80%)	15(1.9%)	< 0.1	1.1	18.8	68.6	96.0
ELISA <i>T.b. gambiense</i>	99.8%	82.8%	900 (90%)	17(1.9%)	< 0.1	0.2	7.5	53.9	95.2
			400 (40%)	4(1.0%)	0.8	21.1	61.6	87.0	96.5
			500 (50%)	5(1.0%)	0.3	16.4	62.2	90.3	98.1
			600 (60%)	7(1.2%)	< 0.1	4.0	41.7	83.5	97.2
			700 (70%)	9(1.3%)	< 0.1	0.9	23.9	75.5	96.8
			800 (80%)	11(1.4%)	< 0.1	0.1	12.3	68.0	96.4
			900 (90%)	13(1.4%)	< 0.1	< 0.1	4.2	58.1	96.0

* HAT = human African Trypanosomiasis; LQAS = lot quality assurance sampling; micro-CATT = micro-card agglutination test for trypanosomiasis; ELISA = enzyme-linked immunosorbent assay.

option with the aim of being able to use a lower cutoff point and thus improving sensitivity. The ELISA is more demanding in terms of equipment, supplies, and staff skills and may therefore not be suitable for use at the level of a regional laboratory. Transporting filter paper samples to a central laboratory is however a feasible option.

A simulation done on the basis of these results and using an LQAS approach showed that theoretically a filter paper based early warning system can reliably distinguish a 2% prevalence at village level from a zero prevalence conditional on testing a sample size of 60% or greater. These findings now need to be verified in a real-life setting.

The use of filter paper samples as a HAT screening tool has been tested before but most earlier studies were based on smaller numbers.^{14–16,21} Laveissière reports on over 24,000 persons tested with micro-CATT but focuses of participation and costs. In Cote d'Ivoire he observed higher participation rates and a 5-fold reduction in costs when compared with the standard screening by a mobile team¹³; whether this also applies in the DRC still needs to be confirmed. Laveissière used general health workers to collect samples within their regular catchment area. Because the samples in our study were collected by mobile teams, we could not perform any meaningful calculations of the actual costs.

Involving general health workers in collecting samples would have added benefits. Apart from the alarm threshold approach, designed to trigger action by a mobile team that should screen the whole village again, the filter paper sample can also be used to identify individual HAT suspects who should then be contacted for diagnostic work-up—at least if the most sensitive test (microCATT) is chosen. This approach has already been piloted in the DRC but was considered impractical because of the long turnaround time of test results. This long turnaround time will be far less of a problem if the process is managed not by mobile teams but by general health workers who are resident in the area. Moreover, involving general health workers can be a meaningful first step toward integration of HAT control into the general healthcare services.

Our study has some limitations. Not all filter paper samples collected contained sufficient quantities of blood to do all tests. This is reflected in the different denominators for each test and each laboratory, and was particularly problematic for the micro-CATT, which requires nine confettis per sample tested. Results for LATEX/*T.b. gambiense* were discarded because of problems related to the batch used.

In low prevalence areas sensitivity cannot be reliably estimated because of the low numbers of HAT cases; in our study we did not identify one single case in the low prevalence area. We therefore calculated sensitivity on the basis of the results of the high prevalence area only. Sensitivity may have been overestimated because all cases were pre-selected based on a positive CATT on whole blood. Our specificity estimates are based on results from the low prevalence area only because the lack of sensitivity of current confirmation algorithms makes it impossible to definitely rule out the presence of false negatives.

The thresholds calculated for raising the alarm are based on the assumption of a random sample. We realize that when using this system to screen village populations the sample will not be truly random but we do not expect major undersampling of infected persons. Oversampling of infected persons would lead to the threshold being surpassed more often at low

prevalence levels. This would not be a major problem because the main distinction to make is between low prevalence and zero prevalence.

In contrast to classical LQAS as applied in vaccination programs, the sample size per lot required in HAT is high because of the low proportions to be distinguished and the lack of a 100% sensitive and specific diagnostic test. The required sample size per village ($\geq 60\%$) is high but not unrealistic given the fact that participation rates in mobile team screening for HAT are in the range of 70% to 80% and vaccination coverages in 2007 in DRC were of 94% for BCG and 95% for DPT-1.²⁹

In conclusion, micro-CATT and ELISA/*T.b. gambiense* are promising candidates as filter paper-based screening tests. Micro-CATT has the added advantage of not requiring sophisticated equipment, making it possible to fully implement the surveillance system at the regional level. Beyond the proof-of-principle provided in this study, much more work needs to be done before a village-based system can be adopted as an alternative to the mobile team surveillance in low prevalence areas. In the next phase of research the most cost-effective surveillance approach needs to be assessed, be it by village-based alarm thresholds followed by exhaustive screening by mobile teams or individual-based diagnostic work-up of those found seropositive. Furthermore, its relative cost and cost-effectiveness compared with the current approach using mobile teams should be properly documented in a real-life setting.

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REFERENCES

1. Brun R, Blum J, Chappuis F, Burri C, 2009. Human African trypanosomiasis. *Lancet* 375: 148–159.
2. World Health Organization, 1998. Control and surveillance of African trypanosomiasis. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser* 881: 1–114.
3. Magnus E, Vervoort T, Van Meirvenne N, 1978. A card-agglutination test with stained trypanosomes (C.A.T.T.) for the serological diagnosis of *T.b. gambiense* trypanosomiasis. *Ann Soc Belg Med Trop* 58: 169–176.
4. Robays J, Bilengue MM, Van der Stuyt P, Boelaert M, 2004. The effectiveness of active population screening and treatment for sleeping sickness control in the Democratic Republic of Congo. *Trop Med Int Health* 9: 542–550.

5. Simarro PP, Jannin J, Cattand P, 2008. Eliminating human African trypanosomiasis: where do we stand and what comes next? *PLoS Med* 5: e55.
6. Lutumba P, Makieya E, Shaw A, Meheus F, Boelaert M, 2007. Human African trypanosomiasis in a rural community, Democratic Republic of Congo. *Emerg Infect Dis* 13: 248–254.
7. Lutumba P, Robays J, Miaka mia Bilenge C, Mesu VK, Molisho D, Declercq J, 2005. Trypanosomiasis control, Democratic Republic of Congo, 1993–2003. *Emerg Infect Dis* 11: 1382–1388.
8. Odiit M, Shaw A, Welburn SC, Fevre EM, Coleman PG, McDermott JJ, 2004. Assessing the patterns of health-seeking behaviour and awareness among sleeping-sickness patients in eastern Uganda. *Ann Trop Med Parasitol* 98: 339–348.
9. Van Nieuwenhove S, Betu-Ku-Mesu VK, Diabakana PM, Declercq J, Bilenge CM, 2001. Sleeping sickness resurgence in the DRC: the past decade. *Trop Med Int Health* 6: 335–341.
10. Lutumba P, Robays J, Miaka C, Kande V, Simarro PP, Shaw AP, Dujardin B, Boelaert M, 2005. The efficiency of different detection strategies of human African trypanosomiasis by *T.b. gambiense*. *Trop Med Int Health* 10: 347–356.
11. Moore A, Richer M, 2001. Re-emergence of epidemic sleeping sickness in southern Sudan. *Trop Med Int Health* 6: 342–347.
12. Laveissiere C, Meda AH, Doua F, Sane B, 1998. Detecting sleeping sickness: comparative efficacy of mobile teams and community health workers. *Bull World Health Organ* 76: 559–564.
13. Cattand P, Jannin J, Lucas P, 2001. Sleeping sickness surveillance: an essential step towards elimination. *Trop Med Int Health* 6: 348–361.
14. Lanata CF, Black RE, 1991. Lot quality assurance sampling techniques in health surveys in developing countries: advantages and current constraints. *World Health Stat Q* 44: 133–139.
15. Miezani T, Doua F, Cattand P, de Raadt P, 1991. Evaluation of Testrpyr CATT applied to blood samples on filter paper and on diluted blood in a focus of trypanosomiasis due to *Trypanosoma brucei gambiense* in the Ivory Coast. *Bull World Health Organ* 69: 603–606.
16. Truc P, Lejon V, Magnus E, Jamonneau V, Nangouma A, Verloo D, Penchenier L, Buscher P, 2002. Evaluation of the micro-CATT, CATT/*Trypanosoma brucei gambiense*, and LATEX/*T.b. gambiense* methods for serodiagnosis and surveillance of human African trypanosomiasis in west and central Africa. *Bull World Health Organ* 80: 882–886.
17. Noireau F, Force-Barge P, Cattand P, 1991. Evaluation of Testrpyr CATT applied to samples of dried blood for the diagnosis of sleeping sickness. *Bull World Health Organ* 69: 603–608.
18. Chappuis F, Pittet A, Bovier PA, Adams K, Godineau V, Hwang SY, Magnus E, Buscher P, 2002. Field evaluation of the CATT/*Trypanosoma brucei gambiense* on blood-impregnated filter papers for diagnosis of human African trypanosomiasis in southern Sudan. *Trop Med Int Health* 7: 942–948.
19. Buscher P, Lejon V, Magnus E, Van Meirvenne N, 1999. Improved latex agglutination test for detection of antibodies in serum and cerebrospinal fluid of *Trypanosoma brucei gambiense* infected patients. *Acta Trop* 73: 11–20.
20. Penchenier L, Grebaut P, Njokou F, Eboo Eyenga V, Büscher P, 2003. Evaluation of LATEX/*T.b. gambiense* for mass screening of *Trypanosoma brucei gambiense* sleeping sickness in Central Africa. *Acta Trop* 85: 31–37.
21. Büscher P, Depla E, Magnus E, Van Meirvenne N, 1995. A serodiagnostic ELISA using variable antigens of *Trypanosoma brucei gambiense*. Sones KR, ed. *22nd Meeting of the ISCTRC*, Kampala, Uganda 25–29 October 1993. Nairobi: OAU/STRC, 46–55.
22. Elrayah IE, Rhaman MA, Karamalla LT, Khalil KM, Buscher P, 2007. Evaluation of serodiagnostic tests for *T.b. gambiense* human African trypanosomiasis in southern Sudan. *East Mediterr Health J* 13: 1098–1107.
23. Woo PT, 1970. The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Trop* 27: 384–386.
24. Lumsden WH, Kimber CD, Evans DA, Doig SJ, 1979. *Trypanosoma brucei*: Miniature anion-exchange centrifugation technique for detection of low parasitaemias: adaptation for field use. *Trans R Soc Trop Med Hyg* 73: 312–317.
25. Lejon V, Buscher P, Magnus E, Moons A, Wouters I, Van Meirvenne N, 1998. A semi-quantitative ELISA for detection of *Trypanosoma brucei gambiense* specific antibodies in serum and cerebrospinal fluid of sleeping sickness patients. *Acta Trop* 69: 151–164.
26. Landis JR, Koch GG, 1977. The measurement of observer agreement for categorical data. *Biometrics* 33: 159–174.
27. Youden WJ, 1950. Index for rating diagnostic tests. *Cancer* 3: 32–35.
28. Tape TG. University of Nebraska Medical Center. *Interpreting diagnostic tests*. Available at: <http://darwin.unmc.edu/dxtests/ROC3.htm>. Accessed April 12, 2010.
29. United Nations International Children's Emergency Fund, 2010. Info by country, statistics. Available at: http://www.unicef.org/infobycountry/drcongo_statistics.html. Accessed January 28, 2010.