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Low Specificities of HIV Diagnostic Tests Caused by *Trypanosoma brucei gambiense* Sleeping Sickness∇

V. Lejon,[1]* D. Mumba Ngoyi,[1,2] M. Ilunga,[3] G. Beelaert,[4] I. Maes,[1] P. Büscher,[1] and K. Fransen[4]

Department of Parasitology, Institute of Tropical Medicine, Antwerp, Belgium[1]; Department of Parasitology, Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo[2]; Programme National de Lutte contre la Trypanosomiase Humaine Africaine (PNLTHA), Mbuji Mayi, East Kasaï, Democratic Republic of the Congo[3]; and Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium[4]

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The accuracy of diagnostic tests for HIV in patients with tropical infections is poorly documented. Human African trypanosomiasis (HAT) is characterized by a polyclonal B-cell activation, constituting a risk for false-positive reactions to diagnostic tests, including HIV tests. A retrospective study of the accuracy of HIV diagnostic tests was performed with 360 human African HAT patients infected with *Trypanosoma brucei gambiense* before treatment and 163 *T. b. gambiense*-infected patients 2 years after successful treatment in Mbuji Mayi, East Kasai, Democratic Republic of the Congo. The sensitivities, specificities, and positive predictive values (PPVs) of individual tests and algorithms consisting of 3 rapid tests were determined. The sensitivity of all tests was 100% (11/11). The low specificity (96.3%, 335/348) and PPV (45.8%, 11/24) of a classical seroconfirmation strategy (Vironostika enzyme-linked immunosorbent assay [ELISA] followed by line immunoassay) complicated the determination of HIV status, which had to be determined by PCR. The specificities of the rapid diagnostic tests were 39.1% for Determine (136/348); 85.3 to 92.8% (297/348 to 323/348) for Vikia, ImmunoFlow, DoubleCheck, and Bioline; and 96.6 to 98.3% (336/348 to 342/348) for Uni-Gold, OraQuick, and Stat-Pak. The specificity of Vironostika was 67.5% (235/348). PPVs ranged between 4.9 and 64.7%. Combining 3 different rapid tests resulted in specificities of 98.3 to 100% (342/348 to 348/348) and PPVs of 64.7 to 100% (11/17 to 11/11). For cured HAT patients, specificities were significantly higher for Vironostika, Determine, Uni-Gold, and ImmunoFlow. *T. b. gambiense* infection decreases the specificities of antibody detection tests for HIV diagnosis. Unless tests have been validated for interference with HAT, HIV diagnosis using classical algorithms in untreated HAT patients should be avoided. Specific, validated combinations of 3 HIV rapid tests can increase specificity.

Over 33 million people worldwide live with HIV, of which two-thirds are in sub-Saharan Africa (16). Rapid diagnostic tests (RDTs) are increasingly being used for HIV diagnosis. In 2007, the proportion of RDTs among diagnostic tests procured through the World Health Organization (WHO) increased to 96%, and the majority were destined for Africa (19). The accuracy of HIV diagnostic tests for persons suffering from common infections in sub-Saharan Africa is poorly documented. In Tanzania, false-positive HIV enzyme-linked immunosorbent assay (ELISA) results were associated with the diagnosis of urinary schistosomiasis and high titers of rheumatoid factor (3). The performance of HIV enzyme immunoassays may be unsatisfactory in patients with visceral leishmaniasis and uncomplicated malaria, resulting in poor positive predictive values (PPVs) (4, 12, 14). These false-positive results of HIV antibody detection tests have been explained by polyclonal B-cell activation in visceral leishmaniasis and malaria (4, 14).

Human African trypanosomiasis (HAT), or sleeping sickness, is a fatal disease caused by the protozoan parasites *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* and is transmitted by tsetse flies. In 36 countries in sub-Saharan Africa where HAT is endemic, about 11,000 new cases are diagnosed yearly from a screened population of 2 to 3 million (15). Polyclonal B-cell activation is also observed in HAT infection (5). The uncontrolled antibody production constitutes a risk for false-positive reactions in diagnostic tests, including HIV tests, but has hardly been studied, although the cross-reaction of antibodies with other pathogens has been reported (1, 2, 13).

We assessed the accuracy of commonly used diagnostic tests to detect antibodies against HIV in patients suffering from *T. b. gambiense* HAT and in a cured subgroup of these patients 2 years after treatment.

MATERIALS AND METHODS

Patients. Three hundred sixty HAT patients infected with *T. b. gambiense* participated in a longitudinal study monitoring clinical outcomes after HAT treatment conducted in the hospital of Mbuji Mayi, Kasai Province, Democratic Republic of the Congo. The patients and study outcomes are described elsewhere (9). Patients were prospectively enrolled according to the following inclusion criteria: (i) tested positive for trypanosomes in lymph node aspirate, blood, or cerebrospinal fluid (CSF); (ii) were ≥12 years old; and (iii) were living within a 100-km perimeter around Mbuji Mayi. Exclusion criteria were as follows: (i) pregnancy; (ii) no guarantee of follow-up; (iii) existence of a moribund condition; (iv) hemorrhagic CSF; and (v) existence of a concurrent serious illness like tuberculosis or bacterial or cryptococcal meningitis. At the time of inclusion, no information was available regarding HIV status. Patients were treated for HAT according to the guidelines of the national program for sleeping sickness of the Democratic Republic of the Congo. Seventeen patients died during treatment,

* Corresponding author. Mailing address: Department of Parasitology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. Phone: 32 3 247 63 69. Fax: 32 3 247 63 73. E-mail: vlejon@itg.be.

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14 died during follow-up for non-HAT-related reasons, 165 experienced treatment failure, and 32 did not appear for the 24-month follow-up visit (9). Cure of HAT, assessed at 24 months posttreatment, was achieved in 163 of 360 patients. The Ministry of Health of the Democratic Republic of the Congo and the Ethical Committee of the University of Antwerp, Belgium, approved the protocol as well as an amendment allowing for HIV testing. We report on results of HIV tests performed retrospectively at the Institute of Tropical Medicine in Antwerp on specimens taken before HAT treatment and on specimens taken at 24 months after HAT treatment from the 163 cured patients.

Specimens. Blood taken on clotting activator was allowed to clot for 1 h at ambient temperature and was centrifuged at 1,000 $\times$ g for 15 min. Serum was collected and immediately frozen in liquid nitrogen, shipped on dry ice, and stored at −80°C until used.

Blood taken on heparin was mixed with an equal volume of AS1 storage buffer (Qiagen, Germany). Specimens were shipped and stored at ambient temperature. DNA was extracted with a QIAamp DNA blood minikit (Qiagen, Germany).

Reference HIV tests. Serum specimens were screened for the presence of HIV-specific antibodies and antigens, using the Vironostika HIV Uni-Form II antigen/antibody ELISA (bioMérieux, Boxtel, Netherlands). If the result of the Vironostika test was reactive (if the optical density was greater than the cutoff value), and taking into account a gray zone (if the cutoff value minus 20% was less than the optical density, and the optical density was less than the cutoff value), the serum was tested with the Inno-Lia HIV I/II score line immunoassay (Innogenetics, Ghent, Belgium). Following the instructions of the manufacturer, Inno-Lia results were interpreted as negative, positive, or indeterminate. The Inno-Lia immunoassay was followed by the Innotest HIV antigen monoclonal antibody ELISA (Innogenetics, Ghent, Belgium) to detect early seroconversions. To confirm or exclude HIV infection in certain cases, HIV PCR was performed according to a nested method in an algorithm of 3 different primer sets in the *pol*, *env*, and long terminal repeat (LTR) regions. The obtained sensitivity and specificity of this PCR was 100%. (17).

Rapid diagnostic tests for detection of HIV-specific antibodies. All serum specimens were tested with the following rapid diagnostic tests according to the manufacturers' instructions: Determine HIV 1/2 (Unipath Limited, Inverness Medical, Bedford, United Kingdom), Uni-Gold HIV (Trinity Biotech, Bray, County Wicklow, Ireland), OraQuick rapid HIV-1/2 antibody (OraSure Technologies, Inc., Bethlehem, PA), DoubleCheckGold HIV 1&2 (Orgenics, Yavne, Israel), SD Bioline HIV-1/2 3.0 (Standard Diagnostics, Inc., Yongin-si, Kyonggi-do, South Korea), ImmunoFlow HIV1-HIV2 (Core Diagnostics, Birmingham, United Kingdom), Vikia HIV 1/2 (bioMérieux, Marcy-L'Etoile, France), and HIV 1/2 Stat-Pak (Chembio Diagnostic Systems, Inc., Medford, NY).

Data analysis. Two-by-two tables were constructed. Stata version 10 software was used to calculate specificities and PPVs with 95% confidence intervals (CI). Specificities were compared, using the McNemar chi-square test.

RESULTS

HIV status of HAT patients before treatment. Stored serum was available for 359 of 360 HAT patients. Determination of the HIV status of the HAT patients was unexpectedly complicated. With Vironostika, 25 of 359 specimens were classified as in the gray zone and 99 of 359 as reactive. With Inno-Lia, 51 of 124 of these specimens tested as indeterminate and 26 of 124 as positive. Eleven Inno-Lia-positive serum specimens reacted strongly with all the HIV-1 test lines (serum glycoprotein 120 [sgp120], gp41, p31, p24, and p17). The remaining 15 Inno-Lia-positive serum specimens reacted weakly and had unusual, incomplete HIV-1 line combinations (there was no reaction with p31, p24, or p17 in 8 to 14 samples). To detect possible seroconverters, all 113 specimens that were in the Vironostika gray zone or Vironostika reactive and Inno-Lia negative, indeterminate, or weakly positive were tested with Innotest HIV antigen, and all were negative. Facing an unusually high number of unclear results with the classical serological confirmation strategy, we performed PCR on Inno-Lia-positive specimens to solve the specificity problem. Only the 11 Inno-Lia strongly positive specimens were confirmed to be HIV positive by PCR.

TABLE 1. Specificities and PPVs with 95% CIs of diagnostic HIV tests in 348 untreated HAT patients

Test	Pretreatment specificities[a] (%; 95% CI)	PPVs[b] (%; 95% CI)
Bioline	323/348 (92.8; 90.1–95.5)	11/36 (30.6; 15.2–45.9)
Determine	136/348 (39.1; 33.9–44.2)	11/223 (4.9; 2.1–7.8)
DoubleCheck	319/348 (91.7; 88.7–94.6)	11/40 (27.5; 13.4–41.6)
ImmunoFlow	314/348 (90.2; 87.1–93.4)	11/45 (24.4; 11.7–37.2)
OraQuick	341/348 (98.0; 96.5–99.5)	11/18 (61.1; 37.9–84.4)
Stat-Pak	342/348 (98.3; 96.9–100)	11/17 (64.7; 41.2–88.2)
Uni-Gold	336/348 (96.6; 94.6–98.5)	11/23 (47.8; 26.9–68.8)
Vikia	297/348 (85.3; 81.6–89.1)	11/62 (17.7; 8.1–27.4)
Vironostika	235/348 (67.5; 62.6–72.5)	11/124 (8.9; 3.8–13.9)

[a] Number of persons with negative test result/total number of persons with HIV-negative status.
[b] Number of persons with HIV-positive status/number of persons with reactive test result.

Thus, HIV prevalence in HAT patients was 3.1% (11/359; CI, 1.5 to 5.4%). The specificity of Vironostika was 67.5% (235/348; CI, 62 to 72%) with a PPV of 8.9% (CI, 4.5 to 15.3%). When samples were considered HIV positive if they were both Vironostika reactive and Inno-Lia positive, the specificity and PPV of the classical serological confirmation strategy were 96.3% (335/348; CI, 94.2 to 98.3%) and 45.8% (11/24; CI, 25.4 to 66.3%), respectively.

Results of HIV RDTs in HAT patients before treatment. The sensitivity of all individual rapid tests was 100% (11/11). The specificities (Table 1) were 39.1% for Determine, 96.6% for Uni-Gold, 98.0% for OraQuick, 91.7% for DoubleCheck, 92.8% for Bioline, 90.2% for ImmunoFlow, 85.3% for Vikia, and 98.3% for Stat-Pak. Positive predictive values (Table 1) ranged from 4.9 to 64.7%.

False positivity seemed randomly scattered, with 1 of 348 HIV-negative samples reactive in 6 RDTs, 2 of 348 in 5 RDTs, 6 of 348 in 4 RDTs, 17 of 348 in 3 RDTs, and 63 of 348 in 2 RDTs.

HIV diagnosis in low-prevalence settings should be based on the combination of 3 different RDTs, and only those patients with 3 reactive assays should be considered HIV positive. All test algorithms consisting of 3 different RDTs resulted in 100% sensitivity; specificities ranged between 98.3% (342/348; CI, 96.9 to 100%; there were 6 false positives) and 100% (348/348). Seventeen specific 3-test combinations provided 100% specificity: Bioline, Determine, and Stat-Pak; Bioline, ImmunoFlow, and OraQuick; Bioline, OraQuick, and Stat-Pak; Bioline, Stat-Pak, and Uni-Gold; Determine, DoubleCheck, and Stat-Pak; Determine, ImmunoFlow, and Stat-Pak; Determine, Stat-Pak, and Vikia; DoubleCheck, ImmunoFlow, and OraQuick; DoubleCheck, ImmunoFlow, and Uni-Gold; DoubleCheck, OraQuick, and Stat-Pak; DoubleCheck, Stat-Pak, and Uni-Gold; ImmunoFlow, OraQuick, and Stat-Pak; ImmunoFlow, OraQuick, and Uni-Gold; ImmunoFlow, Stat-Pak, and Uni-Gold; ImmunoFlow, Uni-Gold, and Vikia; OraQuick, Stat-Pak, and Vikia; and Stat-Pak, Uni-Gold, and Vikia. The order in which the test combinations are presented is alphabetical.

TABLE 2. Results of HIV ELISAs and RDTs for 161 HIV-negative HAT patients before and 2 years after successful treatment for HAT[a]

Test	Pretreatment specificities[b] (%; 95% CI)	Posttreatment specificities[b] (%; 95% CI)	*P* values
Bioline	150/161 (93.2; 89.2–97.1)	154/161 (95.7; 92.5–98.8)	0.25
Determine	74/161 (46.0; 38.2–53.7)	139/161 (86.3; 81.0–91.7)	<0.001
DoubleCheck	148/161 (91.9; 87.7–96.2)	130/161 (80.7; 74.6–86.9)	0.003
ImmunoFlow	147/161 (91.3; 86.9–95.7)	155/161 (96.3; 93.3–99.2)	0.046
OraQuick	158/161 (98.1; 96.0–100)	160/161 (99.4; 98.2–100)	0.16
Stat-Pak	158/161 (98.1; 96.0–100)	160/161 (99.4; 98.2–100)	0.3
Uni-Gold	155/161 (96.3; 93–99.2)	160/161 (99.4; 98.2–100)	0.03
Vikia	134/161 (83.2; 77.4–89.1)	144/161 (89.4; 84.6–94.2)	0.08
Vironostika	119/161 (73.9; 67.1–80.8)	158/161 (98.1; 96.0–100)	<0.001

[a] Specificities were compared by using the McNemar chi-square test.
[b] Number of persons with negative test result/total number of persons with HIV-negative status.

HIV status of cured HAT patients 2 years after HAT treatment. Two years after treatment for HAT, 163 of 360 patients were declared cured of HAT. Serum specimens were available from 162 of these patients. Among these 162 patients, one was HIV positive before HAT treatment, and HIV positivity was reconfirmed by HIV PCR 2 years posttreatment. Among the 161 remaining samples, 4 were in the gray zone or reactive with Vironostika and indeterminate or positive with Inno-Lia. They were negative by Innotest HIV antigen test and PCR. Thus, all 161 HIV pretreatment-negative patients remained HIV negative. The specificity of the Vironostika test increased significantly (Table 2).

Results of HIV RDTs in cured HAT patients. The serum of the HIV-positive patient remained reactive with all HIV RDTs after the patient was cured of HAT. Specificities increased significantly for the Determine, Uni-Gold, and ImmunoFlow tests and were higher, but not significantly higher, for the OraQuick, Bioline, Vikia, and Stat-Pak tests (Table 2). For DoubleCheck, the specificity was significantly lower for specimens from cured patients.

DISCUSSION

When testing *T. b. gambiense* HAT patients, low specificities were observed for antibody detection RDTs as well as for reference tests (ELISA and line immunoassay) for HIV diagnosis. In cured HAT patients, specificities were higher. Sleeping sickness thus decreases the specificities of HIV diagnostic antibody detection tests, including tests and algorithms used for confirmation.

Test specificities of HIV RDTs observed in HAT patients are below the specificities of ≥99% reported by WHO (18). Surprisingly, this is the first report on the low specificities of HIV tests in patients with *T. b. gambiense* HAT. Other studies examining HIV prevalences in HAT patients did not report specificity problems with the second-generation ELISA and Western blot tests used (7, 8, 10, 11). False-positive results of HIV ELISAs were observed for patients with uncomplicated malaria, with specificities of 96.4 and 98.4% and positive predictive values of 53 and 57% (4), and for 9% of patients with visceral leishmaniasis (14). Urinary schistosomiasis and rheumatoid factor were associated with false-positive HIV test results (3). Although *T. b. gambiense* is not the only protozoan parasite that impairs HIV test specificity, its effect seems more profound. This might be due to the strong nonspecific polyclonal B-cell activation and/or high concentrations of rheumatoid factor-like anti-immunoglobulin antibodies that occur in sleeping sickness (5, 6). The specificity differences among tests might be caused by different antigens or by a different involvement of IgM. To increase sensitivity, several third-generation tests include IgM in the reaction. There is a higher risk of nonspecific reactions with such HIV tests, as antibodies resulting from the B-cell activation in HAT belong mainly to the IgM class (5).

In the absence of a negative-control group from a region where HAT is endemic, the relationship between the low test specificities and HAT could be demonstrated by comparing pre- and post-HAT treatment specificities for the same patients. The presence of other immunological disorders or biological factors such as coinfections in our HAT patients were not examined. Although we cannot exclude their interference in the performance of the HIV tests, this seems unlikely since the same tests were repeated in the same persons after they were cured of HAT. The overall specificities of HIV RDTs for patients in the region remain unknown, although they are expected to be equal to or higher than the observed specificities for patients cured of HAT. The accuracy of DoubleCheck-Gold HIV 1&2 should be further investigated, since we have no explanation for its decrease in specificity after successful treatment for HAT despite use of the same test batch number.

Considering the difficulties we encountered at the reference laboratory in determining the real HIV status of HAT patients, it is obvious that the risk for wrong diagnosis is quite high. There is the risk not only of false-positive HIV diagnosis in patients with confirmed HAT but also of HAT being misdiagnosed as HIV, leaving the HAT patient without treatment. Confounding HAT and HIV diagnoses may become particularly problematic in the context of the integration of HAT control in basic health infrastructures (15). Clinicians easily oversee HAT if they are not familiar with the disease, since the clinical picture may mimic malaria, tuberculosis, toxoplasmosis, viral encephalitis, brucellosis, lymphoma, typhoid fever, neurosyphilis, HIV, and opportunistic infections (e.g., cryptococcosis or toxoplasmosis). One should be careful to generalize our observations, since we have not tested the accuracy of HIV antibody tests for *T. b. rhodesiense* HAT patients.

Our results support the recommendation by WHO to perform 3 different RDTs for HIV diagnosis in settings with prevalences lower than 10% (18). Considering only those persons HIV positive who are reactive with 3 different tests in-

creases the total specificity of HIV RDTs for HAT patients, although up to 1.7% false positivity remained. Although we report on the specificities of different RDTs and of test algorithms for HIV diagnosis for *T. b. gambiense* HAT patients, we withhold from proposing an optimal test algorithm for regions where *T. b. gambiense* is endemic. In the present study, the number of HIV-positive samples was too low to make reliable estimations of test sensitivities. The performances of the various assays, both reference tests and RDTs, remain to be determined in a HAT-negative population. Moreover, besides test sensitivity and specificity, other factors influence the choice of the most suitable test algorithm, such as cost, test availability, robustness, user friendliness, test rapidity, and necessary equipment.

In conclusion, *T. b. gambiense* infection decreases the specificity of antibody detection tests for HIV diagnosis. Even a gold standard seroconfirmation strategy does not provide a solution for determining the real HIV status, and limited data on the performance of a Western blot test also show lower specificity caused by indeterminate results. Since the risk for HIV misdiagnosis is considerable, HIV diagnosis should not be established in the setting of an untreated HAT infection by using classical algorithms unless the applied tests have been validated specifically for noninterference with HAT. Although a risk for false-positive reactions for HAT patients remains, specific combinations of 3 different serially applied HIV RDTs increase specificity and should be applied for the determination of HIV status.

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