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

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Objective To evaluate the performance of serological tests using dried blood on filter-papers (micro-card agglutination test for trypanosomiasis (micro-CATT)) performed under field and laboratory conditions and using whole blood ((CATT/*T.b. gambiense*) (wb-CATT) and latex agglutination (LATEX/*T.b. gambiense*) (wb-LATEX)) for the serodiagnosis and surveillance of human African trypanosomiasis in West and Central Africa.

Methods We evaluated the micro-CATT, wb-CATT and wb-LATEX methods in Côte d'Ivoire and the Central African Republic by screening 940 people. Sensitivity and specificity were calculated for each serological test; only patients with the confirmed presence of trypanosomes in the blood or lymph aspirate were considered true positives. Positive and negative predictive values were also calculated.

Findings Each of the tests showed a lower sensitivity in the Central African Republic than in Côte d'Ivoire.

Conclusion The results confirmed the efficiency of the classic wb-CATT to detect sleeping sickness patients. The micro-CATT method can be used for human African trypanosomiasis surveillance if the test is performed on the same day as the blood collection, or if samples are stored at 4 °C. Otherwise, micro-CATT can be used when absolute sensitivity is not required. wb-LATEX should only be used for high-specificity screening.

Key words Agglutination tests; Sensitivity and specificity; Predictive value of tests, Trypanosomiasis, African/diagnosis; Comparative study; Côte d'Ivoire; Central African Republic (*source: MeSH, NLM*).

Mots clés Réaction agglutination; Sensibilité et spécificité (Epidémiologie); Valeur prédictive tests; Trypanosomiase africaine/diagnostic; Etude comparative; Côte d'Ivoire; Républic centrafricaine (*source: MeSH, INSERM*).

Palabras clave Tests de aglutinación; Sensibilidad y especificidad; Valor predictivo de los tests; Tripanosomiasis africana/diagnóstico; Estudio comparativo; Côte d'Ivoire; República Centroafricana (*fuente: DeCS, BIREME*).

Bulletin of the World Health Organization 2002;80:882-886.

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Introduction

Human African trypanosomiasis remains a major health problem in sub-Saharan Africa, where about 60 million people are exposed daily to infecting tsetse fly bites. In 1998 a total of 40 000–45 000 new cases were reported. Despite this, only 3 million people at risk are under surveillance. WHO estimates that about 500 000 persons are infected with human African trypanosomiasis (*1*), a level that permits active cyclical transmission of the disease, as confirmed by current epidemics in Central Africa.

In order to follow the spread of the disease within historical foci and to detect emerging foci, national control programmes should increase epidemiological surveillance. In 1995, WHO initiated an international human African trypanosomiasis surveillance programme in 18 countries in West and Central Africa, where the chronic form of the disease due to *Trypanosoma brucei gambiense* occurs. One of the main objectives was to stimulate national control programmes to set up active serological surveillance of the disease among their populations. WHO recommended the use of a serological test (micro-card agglutination test for trypanosomiasis) (micro-CATT) (*2*), a variant of the card agglutination test for trypanosomiasis (CATT/*T.b. gambiense*) used with whole blood (wb-CATT) (*3*). The micro-CATT is considered to be inexpensive, easy to use under field conditions, and allows the collection and storage of dried blood samples on filter-paper for future serological analysis.

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Ref. No. **01-1368**

The major objectives of the study were to determine the sensitivity and specificity of the micro-CATT using dried blood and to compare the results with the sensitivities and specificities obtained using two other card agglutination tests for detecting antibodies in whole blood (wb-CATT) and the latex agglutination test (LATEX/*T.b. gambiense*) (wb-LATEX). The study was carried out in two epidemiological and geographical situations: Batangafo (Central African Republic), and Bonon (Côte d'Ivoire). Because of the difficulty of performing micro-CATT under field conditions, the micro-CATT results were compared using the same filter-papers under field or laboratory conditions.

Methods

Study areas and populations

The study was carried out in 1999 during two medical surveys and included 940 consenting persons. The first survey was conducted in Batangafo, in the north of Central African Republic, by the National Control Programme of the Central African Republic and the human African trypanosomiasis team of the Organisation de Coordination pour la Lutte contre les Endémies en Afrique Centrale (OCEAC), Yaoundé, Cameroon; and the second in Côte d'Ivoire, in the Bonon area, 80 km west of Bouaflé, by the Institut Pierre Richet, Bouaké, Côte d'Ivoire. In Batangafo, 540 people were examined; and in Bonon, 400 people.

Sample collection

To avoid multiple finger-pricks, samples of venous blood were obtained from each individual by venepuncture using two 5-ml Venoject® tubes (available from VWR International, Darmstadt, Germany). One tube without anticoagulant was used for immediate tests, including the standard field protocol of the micro-CATT (*2*) and the parasitological quantitative buffy coat (QBC) test (*4*). The whole blood was quickly transferred to a QBC tube coated with heparin, which was then centrifuged and examined under the microscope for trypanosomes. The remaining whole blood from this sample was adsorbed onto four 20 cm^2 sheets of filter-paper (Whatman No. 4) for subsequent micro-CATT tests. Micro-CATT tests were performed at three times: in the field on the day of sample collection (day 0); on day 3; and on day 7. To evaluate the influence of time and storage conditions of the samples on these results, we divided the dried blood samples into equal aliquots and stored them either at local room temperature or refrigerated.

A second tube containing an anticoagulant — ethylenediaminetetraacetic acid (EDTA) — was used to collect blood for immediate serological tests by wb-CATT on whole blood. Some of the blood was also stored on four sheets of Whatman No. 4 filter-paper and the samples sent to the Institute of Tropical Medicine, Antwerp, Belgium, for further analysis. EDTA was used as a coagulant instead of heparin to avoid the prozone effect, previously observed when performing wb-CATT with heparin-treated samples (*5*). All dried blood samples collected on the filter-paper were stored in glass containers containing silica gel at 4 °C. The protocol for micro-CATT was modified at the Institute for Tropical Medicine, as described below. During the study, the same serological and parasitological tests were used, as well as the same batches of filter-paper and serological reagents.

Serological and parasitological tests in the field

The tests described below were performed immediately after collection of the blood samples (day 0):

- wb-CATT (*3*) using whole blood according to the instructions of the manufacturer (Institute of Tropical Medicine, Antwerp, Belgium). No CATT tests were performed on plasma or serum.
- wb-LATEX (*6, 7*) a card agglutination test, similar to wb-CATT, but the reagent is a mixture of three purified variable surface glycoproteins (LiTat 1.3, 1.5, and 1.6) adsorbed on latex beads. The reagent was provided desiccated and reconstituted with 2 ml of phosphate-buffered saline. The reagent solution (40 μl) was then dispensed on each reaction zone of a plastic card, and 5 μl of blood was mixed together with the reagent on each reaction zone. Subsequently, the card was placed on a flat-bed rotator (Type B2, Institute of Tropical Medicine, Antwerp, Belgium) and agitated at 70 rpm for 5 minutes. The agglutination was read macroscopically.
- On day 0, the field micro-CATT was performed according to a previously described protocol (*2*).
- The QBC test was carried out using a modified protocol (*4*).
- A cervical palpation was performed on each individual to detect lymphadenopathy. If present, a puncture was performed and the aspirate examined by light microscope for parasites.
- The micro-CATT was repeated on day 3 and on day 7 on dried blood samples from individuals with positive results for at least one of the serological tests, wb-CATT, micro-CATT (day 0), or wb-LATEX. The same dried blood samples used for the day 0 test were used for the repeat tests. Filter-paper samples were stored at local room temperature (described above).

For all serological tests (micro-CATT, wb-CATT, wb-LATEX), agglutination was considered to be a positive result (*8*).

Serological test in the laboratory

At the Institute of Tropical Medicine, Antwerp, micro-CATT was performed on EDTA-treated blood samples eluted from dried filter-papers at least 6 weeks after sample collection, using a modified protocol from that originally described (*2*). For each blood sample, disks (6 mm) were punched out of areas of the filter-papers with dried blood, placed into one well of a 96-well flat-bottomed microplate (Greiner Bio-One, Longwood, FL, USA), and the blood extracted with 30 μl CATT buffer for 1 hour at room temperature (20–25 °C) with gentle shaking every 15 minutes. CATT reagent was reconstituted by adding 1.25 ml of CATT buffer, instead of the usual 2.5 ml in order to concentrate the reagent. Sample eluate (10 μl) was then mixed with 5 μl of concentrated CATT reagent on the reaction zones (10-mm diameter) of the test cards. The card was placed on a flat-bed rotator (Type B2, Institute of Tropical Medicine, Antwerp, Belgium) and agitated for 7 minutes at 70 rpm. The agglutination patterns were read immediately using a X 3.5 magnifying glass. The same lot of Whatman filter-paper and the same batch of serological reagents were used to collect and test all field and laboratory samples. The laboratory personnel were not aware of results we had obtained in the field.

Statistical analysis

The following statistical parameters were calculated for each serological test: sensitivity, specificity, positive predictive value

(PPV), and negative predictive value (NPV). 95% symmetrical confidence intervals of sensitivity and specificity were calculated using JavaStat. Only patients in whom trypanosomes had been confirmed in blood or lymph aspirates were considered to be true positives. Since the parasitological reference tests (QBC and detection of trypanosomes in lymph aspirate) were not 100% sensitive (*4*), cases of false seropositivity were likely. Thus, the observed specificity should be considered minimal. Also, some seronegative individuals may have been infected, which would lead to an overestimation of the observed sensitivity. Sensitivities and specificities of the serological tests were all compared by the McNemar χ^2 test (one degree of freedom with continuity correction).

Results

Based on parasite detection either in the blood (QBC) or lymph aspirate, 66 individuals were diagnosed to have human African trypanosomiasis: 56 in Batangafo, and 10 in Bonon. All patients were treated by the local medical staff according to the national drug policy, as recommended by WHO (*1*).

Table 1 shows the sensitivity, specificity, PPV, and NPV of the different serological tests in Central African Republic and Côte d'Ivoire. In Batangafo, 16 of 56 patients had trypanosomes in their lymph aspirates, 50 were micro-CATT positive on day 0, 55 were wb-CATT positive, and 38 were wb-LATEX positive. Of 484 persons with no detected trypanosomes, 32 were micro-CATT positive on day 0, 29 were wb-CATT positive, and 3 wb-LATEX positive (false-positive cases). At the Institute of Tropical Medicine, Antwerp, blood samples from 53 of 56 patients from the Central African Republic were found to be micro-CATT positive. Of the 484 people in whom trypanosomes were not detected, 16 were micro-CATT positive, but were not confirmed parasitologically.

In Bonon, trypanosomes were detected in lymph aspirates from 4 of 10 patients, 9 patients were micro-CATT positive on day 0, 10 patients were wb-CATT positive, and 9 were wb-LATEX positive. Of the 390 persons with no detectable trypanosomes, 7 were micro-CATT positive on day 0, 13 were wb-CATT positive, and 4 were wb-LATEX positive (false-positive cases). All 10 patients from Bonon were found to be micro-CATT positive by the laboratory in Antwerp. Of 390 people with no detectable trypanosomes, 14 were micro-CATT positive (false-positive cases).

The wb-LATEX method was significantly less sensitive (P = 0.0001–0.019) and more specific than all other tests (P<0.0001). The micro-CATT performed in the field was significantly less sensitive than the wb-CATT (89.4% vs 98.5%, P = 0.0412), but both had the same specificity. We found no significant difference in sensitivity and specificity between micro-CATT performed in the field and in Antwerp. Micro-CATT performed in Antwerp was, however, more specific than wb-CATT (96.6% vs 95.2%, P = 0.0485).

The micro-CATT was repeated three and seven days after blood collection for all parasitologically confirmed patients, since the single patient from Batangafo who was wb-CATT negative was positive by the wb-LATEX assay. The sensitivity of the micro-CATT at days 0, 3, and 7 is shown in Table 2. The overall sensitivity decreased from 89.4% to 50% when filter-papers were stored for 7 days at room temperature.

Discussion

The sensitivity of all serological tests used in this study was higher in Bonon (Côte d'Ivoire) than in Batangafo (Central African Republic). This confirms previous results obtained in the northern foci of the Democratic Republic of Congo and in the Republic of Congo (*7*), where both wb-CATT and wb-LATEX were found to be less sensitive than in Côte d'Ivoire (*8*). One explanation for this is that the main antigenic variants used in the serological tests (LiTat 1.3, 1.5 and 1.6) are not the predominant antigenic types expressed by *T.b. gambiense* in Central Africa. Indeed, it was found that the *LiTat 1.3* gene was missing from some strains of trypanosomes isolated in Cameroon (*9*).

Table 1. **Sensitivity and specificity of micro-CATT, CATT/*T. b. gambiense* and LATEX/*T. b. gambiense* in Batangafo (Central African Republic) and Bonon (Côte d'Ivoire)[a]**

Test	Population	Sensitivity	PPV[b] (%)	Specificity	NPV[c] (%)
micro-CATT field	Batangafo	50/56 (89.3)[d]	61.0	452/484 (93.4)	98.7
	Bonon	9/10 (90.0)	56.3	383/390 (98.2)	99.7
Total		**59/66 (89.4);** ***79.4–95.6***[e]		**835/874 (95.5);** ***94.0–96.8***	
micro-CATT, ITMA	Batangafo	53/56 (94.6)	76.8	468/484 (96.7)	99.4
	Bonon	10/10 (100.0)	41.7	376/390 (96.4)	100
Total		**63/66 (95.5);** ***87.3–99.1***		**844/874 (96.6*);95.1–97.7***	
wb-CATT field	Batangafo	55/56 (98.2)	65.5	455/484 (94.0)	99.8
	Bonon	10/10 (100.0)	43.5	377/390 (96.7)	100
Total		**65/66 (98.5);** ***91.8–100***		**832/874 (95.2);** ***93.6–96.5***	
wb-LATEX field	Batangafo	38/56 (67.9)	92.7	481/484 (99.4)	96.4
	Bonon	9/10 (90.0)	69.2	386/390 (99.0)	99.7
Total		**47/66 (71.2*); 58.8–81.7***		**867/874 (99.2*); 98.4–99.7***	

[a] The micro-CATT test was performed in the field on day 0 (micro-CATT field), or at the Institute of Tropical Medicine, Antwerp (micro-CATT, IMTA). Both the CATT/*T. b. gambiensis* test (wb-CATT field) and the LATEX/*T. b. gambiensis* test (wb-LATEX field) were performed in the field using whole blood.

[b] PPV = Positive predictive value.

[c] NPV = Negative predictive value.

[d] Figures in parentheses are percentages.

[e] Figures in italics are 95% confidence intervals.

Table 2. **Effects on sensitivity of storing micro-CATT filter-papers at room temperature for 0, 3 and 7 days**[a]

Focus	Sensitivity		
	Day 0	Day 3	Day 7
Batangafo, Central African Republic	50/56 (89.3)[b]	38/56 (67.8)	26/56 (46.4)
Bonon, Côte d'Ivoire	9/10 (90.0)	9/10 (90.0)	7/10 (70.0)
Total	**59/66 (89.4)**	**47/66 (71.2)**	**33/66 (50)**

[a] The tests were performed in the field on patients with confirmed parasite infections.
[b] Figures in parentheses are percentages.

Under field conditions, the micro-CATT at day 0 had sufficient sensitivity and specificity in both Central African Republic and Côte d'Ivoire. However, the 3- and 7-day results indicated that storing filter-papers at local room temperature for one week led to a rapid decrease in sensitivity. Despite this, we found no significant difference in performance (sensitivity or specificity) between the micro-CATT performed in the field (day 0), or in the laboratory (6 weeks after sample collection), indicating that results are influenced by the storage condition of samples. Therefore, samples must be kept at 4 °C until use.

The micro-CATT appears to be suitable for the serodiagnosis of human African trypanosomiasis when performed on the day of sample collection or when the blood samples are kept at 4 °C, which confirms previous results (*2, 10*). However, such stringent conditions are unrealistic in the field. If blood samples have to be tested immediately after collection, there would be no point in performing a micro-CATT rather than a wb-CATT. Moreover, the highest sensitivity was obtained when the wb-CATT was performed in the field — this test was significantly more sensitive than micro-CATT and wb-LATEX tests in the field — and had a similar specificity to the micro-CATT assay performed in the field on day 0. While the micro-CATT might be preferred for logistical or technical reasons, it is more expensive than the wb-CATT test, even if less reagent is required. Indeed, both tests require the same basic equipment (rotator, refrigerator), but the micro-CATT requires additional consumables (Whatman No. 4 filter-paper, silica gel for storage, 96-well microplates).

While it may be possible in some areas to refrigerate dried blood samples, our experience has been that when blood collection is performed by community health workers, samples are generally stored at local room temperature and are often in the field for several days before reaching the laboratory for the micro-CATT test. As demonstrated by the present study, this leads to an important decrease in test sensitivity. However, the micro-CATT could still be considered for HAT surveillance when optimal sensitivity is not required, or when it is difficult for the medical teams to access the infection foci.

Although the wb-LATEX assay was less sensitive than the other tests, it was more specific. In Batangafo, the low sensitivity of the wb-LATEX compared to wb-CATT may be explained by differences in antigen composition. wb-LATEX is based on a mixture of LiTat 1.3 (the same as the wb-CATT), LiTat 1.5, and LiTat 1.6 antigens (*6, 7*). The main reacting epitopes are variable, with some invariant epitopes on the rest of the variable surface glycoprotein molecules. In the wb-CATT reagent, by contrast, the variable surface glycoproteins bearing simultanously highly specific variable and less specific invariable epitopes and other invariable antigens can contribute to a positive reaction (agglutination). In addition, if only anti-LiTat 1.3 antibodies are present in the test sample, the reaction obtained using wb-CATT will be stronger than that obtained with the wb-LATEX test, since in the wb-LATEX reagent LiTat 1.3 antigens comprise only one-third of the total antigens. Consequently, wb-LATEX assays, performed as recommended by this study, should only be used for high-specificity testing in West Africa.

In conclusion, we confirm that wb-CATT can efficiently detect sleeping sickness patients in both West and Central Africa. In the absence of other cheap and simple techniques, it remains the reference test for control activities under field conditions. The micro-CATT assay can be used for HAT surveillance when high sensitivity is not essential. Finally, it is recommended that the wb-LATEX assay be used only for high-specificity testing in West Africa, but not in Central Africa. ■

Acknowledgements

We would like to express our sincere thanks to the teams of the human African trypanosomiasis National Control Programme of the Central African Republic, of OCEAC (Cameroon), and of the Institut Pierre Richet, Côte d'Ivoire, for their excellent technical help. We thank Mr Pierre Cattand and Dr L.K. Basco (OCEAC, Yaoundé, Cameroon) for their constructive feed-back on earlier drafts of this paper. This work was supported by the Fonds d'Aide à la Coopération, Ministère Français des Affaires Etrangères, de la Coopération et de la Francophonie, and by the Institut de Recherche pour le Développement, France.

Conflicts of interest: none declared.

Résumé

Evaluation des méthodes micro-CATT, CATT/*Trypanosoma brucei gambiense* et LATEX/*T. b. gambiense* pour le sérodiagnostic et la surveillance de la trypanosomiase humaine africaine en Afrique de l'Ouest et en Afrique centrale

Objectif Evaluer la performance de tests sérologiques utilisant du sang séché sur papier filtre (micro-test d'agglutination sur carte pour la trypanosomiase (micro-CATT)) réalisés sur le terrain et en laboratoire, et de tests utilisant du sang total (CATT/*T. b. gambiense* (wb-CATT)) et agglutination sur latex (LATEX/*T. b. gambiense* (wb-LATEX)) pour le sérodiagnostic et la surveillance de la trypanosomiase humaine africaine en Afrique de l'Ouest et en Afrique centrale.

Méthodes Nous avons évalué les méthodes micro-CATT, wb-CATT et wb-LATEX en Côte d'Ivoire et en République centrafricaine en procédant à un dépistage sur 940 personnes. La sensibilité et la spécificité ont été calculées pour chaque test ; seuls les patients chez qui la présence de trypanosomes dans le sang ou le suc ganglionnaire était confirmée ont été considérés comme vrais positifs. Les valeurs prédictives positive et négative ont également été calculées.

Résultats Tous les tests présentaient une plus faible sensibilité en République centrafricaine qu'en Côte d'Ivoire.

Conclusion Les résultats ont confirmé l'efficacité du wb-CATT classique pour détecter les patients atteints de maladie du sommeil. La méthode micro-CATT peut être utilisée pour la surveillance de la trypanosomiase humaine africaine si le test est réalisé le jour même du prélèvement de sang ou si les échantillons sont conservés à 4 °C. Sinon, le micro-CATT peut être utilisé lorsqu'il n'est pas nécessaire d'avoir une sensibilité absolue. Le test wb-LATEX ne doit être utilisé que pour les dépistages hautement spécifiques.

Resumen

Evaluación de los métodos micro-CATT (CATT/*Trypanosoma brucei gambiense* y LATEX/*T. b. gambiense*) como medio de serodiagnóstico y vigilancia de la tripanosomiasis africana humana en África occidental y central

Objetivo Evaluar el funcionamiento de las pruebas serológicas basadas en la aplicación de sangre seca en papel de filtro (prueba de aglutinación en microtarjetas para la tripanosomiasis (micro-CATT)), sobre el terreno y en el laboratorio, utilizando sangre entera (CATT/*T.b. gambiense* (wb-CATT)) y aglutinación en látex (LATEX/*T.b. gambiense* (wb-LATEX)), con fines de serodiagnóstico y vigilancia de la tripanosomiasis africana humana en África occidental y central.

Métodos Los métodos micro-CATT, wb-CATT y wb-LATEX se evaluaron sometiendo a cribado a 940 personas en Côte d'Ivoire y en la República Centroafricana. Se calcularon la sensibilidad y la especificidad de cada prueba serológica; sólo se consideraron verdaderos positivos los pacientes con presencia confirmada de tripanosomas en la sangre o el aspirado linfático. Se calcularon también los valores predictivos positivo y negativo.

Resultados En cada una de las pruebas la sensibilidad fue menor en la República Centroafricana que en Côte d'Ivoire.

Conclusión Los resultados confirmaron la eficiencia de la prueba tradicional de wb-CATT como medio de detección de los pacientes con enfermedad del sueño. El método micro-CATT puede utilizarse para vigilar la tripanosomiasis africana humana si la prueba se realiza el mismo día de obtención de la sangre, o si las muestras se almacenan a 4 °C. En caso contrario, también es posible utilizar esa técnica si no se requiere una sensibilidad absoluta. La prueba wb-LATEX sólo debe utilizarse en las iniciativas de cribado que exijan una alta especificidad.

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