**Detection of trypanosome-specific antibodies in saliva, towards non-invasive serological diagnosis of sleeping sickness**

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**Summary**

**OBJECTIVE** The detection of trypanosome-specific antibodies in saliva is technically feasible, and, if clinically validated, could become an attractive option for non-invasive diagnosis of sleeping sickness.

**METHODS** Different ELISA formats for antibody detection in serum and saliva were developed and standardized. Saliva and serum samples were collected from 78 patient and 128 endemic control samples, and sensitivity and specificity of saliva ELISAs, serum ELISAs and the card agglutination test for trypanosomiasis (CATT), were evaluated.

**RESULTS** All ELISA formats showed sensitivity and specificity above 90%. Saliva ELISAs showed a similar test performance as serum ELISAs and the CATT on whole blood or serum.

**CONCLUSIONS** This study confirms the potential of trypanosome-specific antibody detection in saliva.

**keywords** human African trypanosomiasis, antibody detection, saliva, serum, diagnosis, *Trypanosoma brucei gambiense*

**Introduction**

Control of sleeping sickness or human African trypanosomiasis is based on diagnosis and treatment of infected patients, usually organized in active population screening campaigns. Because of the high workload generated by parasitological examinations in mass screening, they are preceded by antibody detection tests in a first screening step. The serological test most often used for this purpose is the card agglutination test for trypanosomiasis (CATT; Magnus et al. 1978), a simple and fast agglutination test that detects antibodies to *Trypanosoma brucei gambiense*. Depending on the geographical region, the reported sensitivity of the CATT is between 87% and 98%, the specificity around 95% (Chappuis et al. 2005). The CATT requires a blood sample, usually obtained by finger prick. This procedure can be difficult to perform especially in children, is not entirely without risk, and is sometimes refused for religious or cultural reasons. Therefore, high priority is given to the development and validation of non-invasive tests (WHO/TDR 2001).

Saliva collection being non-invasive and painless, could represent an attractive alternative for the current screening method that is based on blood sampling. In a pilot study on a limited number of sleeping sickness patients and non-endemic healthy controls, we demonstrated that the detection of trypanosome-specific antibodies in saliva of sleeping sickness patients is feasible (Lejon et al. 2003). In this study a simple enzyme-linked immunosorbent assay (ELISA) was used, in which the saliva sample incubation step was directly followed by a conjugate incubation step. As a consequence of the limited analytical sensitivity of this ELISA and the low antibody concentrations present in saliva, optical densities of some patient samples were close to the optical densities of negative control saliva. Moreover, evaluation of positivity of the saliva samples in ELISA was based on the absolute value
of the corrected optical density (OD), compared with a cut-off. This reduces the reproducibility of the test, because the OD can be influenced by external factors such as temperature, incubation time and the batch of reagents used to perform the test.

Our objectives in this study were to further evaluate the potential of antibody detection in saliva for diagnosis of sleeping sickness on a larger sample group, including endemic controls. Therefore, we developed an indirect ELISA test format with increased analytical sensitivity that enhanced the detection of specific antibodies in serum and saliva by using a secondary antibody against human immunoglobulin (Ig)G. This study reports the performance of different ELISA formats on saliva, serum, and the CATT on serum and blood. The latter was used as the current reference standard for a field test. Finally, results obtained with the different ELISA formats on saliva and serum were compared in order to choose the test with the highest diagnostic accuracy (highest sensitivity, specificity and Youden index).

Materials and methods

Study population

Paired serum and saliva samples were collected in a sleeping sickness epidemic zone in D. R. Congo and in a hypoendemic zone in Benin. In Congo, samples of 78 T. b. gambiense patients and 47 negative controls were collected in the trypanosomiasis treatment centres of Kwamouth (Bandundu province) and Maluku (Kinshasa) and at the Institut National de Recherche Biomédicale (INRB Kinshasa) between July and December 2004. All T. b. gambiense patients were CATT-seropositive on whole blood (Magnus et al. 1978) and trypanosomiasis infection was confirmed parasitologically by examination of blood [stained thick drop examination, haematocrit centrifugation technique and/or mini anion-exchange centrifugation technique (Woo 1971; Lumsden et al. 1979)], lymph node fluid or cerebrospinal fluid (CSF; direct examination in cell counting chamber). Negative controls had a negative CATT on whole blood (CATT-wb), showed no symptoms indicative of sleeping sickness and were never treated for the disease. No parasitological tests were performed on these persons.

In Benin, samples of 81 individuals were collected in the historical Atakora focus (Avode et al. 1988) during an active screening in May 2004. Nineteen were CATT-wb positive and were tested for the presence of parasites by the mini anion-exchange centrifugation technique (Lumsden et al. 1979) and the Quantitative Buffy Coat technique (Bailey & Smith 1992). In none of these persons parasites could be found. The other 62 persons were negative in CATT-wb, did not show symptoms indicative for sleeping sickness and were never previously treated for the disease. They were not tested parasitologically.

Sample collection and processing

Blood was taken intravenously and serum was prepared according to standard protocols. Saliva samples were collected with the Salivette system (Sarstedt). A cotton plug was held for 5 min under the tongue. Saliva was eluted from it by centrifugation in the specially designed tube. Serum and saliva were frozen, sent to the Institute of Tropical Medicine (ITM), Belgium on dry ice and stored at −80 °C. Before testing at ITM, samples were centrifuged for 5 min at 2900 g.

CATT/T. b. gambiense on whole blood and on serum

The card agglutination test for trypanosomiasis on whole blood was performed in the field, CATT on serum dilutions 1:4 and 1:8 at ITM. Both tests were performed according to the instructions of the manufacturer and scored with the accompanying reference card (Magnus et al. 1978). The test was considered positive when the agglutination was scored + or stronger. For serum, a person was considered positive when a positive reaction occurred at a serum dilution of 1:4 or higher.

Direct ELISA/T. b. gambiense

Antibodies in serum and saliva were detected by the direct ELISA/T. b. gambiense as described earlier (Büscher et al. 1994; Lejon et al. 2003), with small modifications. Microplates (Maxisorp, Nunc) were coated overnight at 4 °C with 150 μl per well of a mixture of purified variable surface glycoproteins of T. b. gambiense LiTat 1.3, LiTat 1.5 and LiTat 1.6 (Büscher et al. 1999) each at a concentration of 2 μg/ml, giving a total protein concentration of 6 μg/ml in phosphate-buffered saline (PBS; 0.01 M phosphate, 0.14 M NaCl, pH 7.4). Antigen-free control wells received 150 μl per well of PBS. Plates were blocked for 1 h at ambient temperature with 350 μl per 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, serum was diluted 1:150 in PBS-Blotto, and saliva was diluted 1:4 and 1:8 in PBS-Blotto. Antigen-containing and antigen-free wells were filled with 150 μl of serum in duplicate or 150 μl of each saliva dilution, and were incubated for 1 h at ambient temperature. Plates were washed three times with 350 μl per well of PBS-Tween (0.01 M phosphate, 0.14 M NaCl, 0.05% w/v Tween 20, pH 7.4) using an automated ELISA washer (ELx50,
Bio-Tek Instruments). Goat antihuman IgG (H + L) peroxidase (Jackson) was diluted in PBS-Tween to a final concentration of 1:80 000 for serum testing and 1:20 000 for saliva testing, and incubated for 60 min (150 μl per well). After five washes, wells were incubated for 1 h at ambient temperature with 150 μl 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) substrate-chromogen solution. The latter was prepared from 50 mg ABTS (Boehringer) dissolved in 100 ml of ABTS buffer (phosphate-citrate-sodium perborate solution, pH 4.6; Boehringer). The plate was shaken for 10 s and the OD was read at 415 nm (Multiskan RC, Version 6.0, Labsystems). Corrected OD values were obtained by subtracting the OD of the antigen-free control well from the OD of the corresponding antigen-containing well. For serum, the average value of the two tested dilutions was taken.

Indirect ELISA/T. b. gambiense

The test protocol of the indirect ELISA/T. b. gambiense was based on an existing ELISA developed for trypanosome-specific IgG detection in serum and CSF (Lejon et al. 1998) with some adaptations. Plates were coated and blocked as for the direct ELISA. Serum samples were diluted 1:15 000 and 1:30 000 in PBS-Blotto, saliva was diluted 1:20 and 1:40 in PBS-Blotto. Antigen-containing and antigen-free wells were filled with 150 μl of serum or saliva dilution, and were incubated for 30 min at ambient temperature with continuous shaking (Cooke AM69 Microshaker). Plates were washed 3 times with 330 μl per well of PBS-Tween and incubated with 150 μl per well of rabbit antihuman IgG (CLB, the Netherlands) diluted 1:4000 in PBS-Blotto. After 1 h, plates were washed three times and were then incubated for 30 min with goat antirabbit IgG (H + L chain) peroxidase (Nordic), diluted 1:20 000 in PBS-Tween. After five washes, the reaction was developed as described above and the corrected OD for each dilution of the samples was calculated.

Standardization of ELISA readings

To improve standardization, all ELISA results were expressed as percentage positivity (PP) relative to a positive control, which was run in each plate. For the ELISA versions testing serum, a positive serum originating from a parasitologically positive patient was run in duplicate in each plate. The positive control was treated in the same way as the test sera. For the ELISA versions testing saliva, the same serum sample was pre-diluted 1:250 to serve as a positive control. The positive control was further diluted as the saliva samples, and was run in each plate in duplicate. The corrected ODs of the positive control in each plate were calculated and the average of the two results was taken. The PP of the test samples was calculated as PP = (Corrected OD test sample/average corrected OD positive control) × 100. The average corrected OD of the positive control in each plate was also used to construct Levey-Jennings charts (Jacobson 1998). This method allows quality control of individual ELISA runs by plotting the average of the ODs of the control sample obtained in different runs (typically 20) with one, two and three times the standard deviation. The OD of individual control runs are plotted in the chart and compared with the standard deviation. When it exceeds the average ±2 SDs, this is considered as a warning for bad quality of the run, which can be rejected if additional criteria apply (Westgard et al. 1981).

Data analysis

The sensitivity, specificity and Youden index (Youden index = sensitivity + specificity − 1, Youden 1950) were calculated for all serological tests. For calculation of the sensitivity, only patients with parasitologically confirmed infection were considered (78 patients from Congo). Calculation of the specificity was based on all 128 individuals without trypanosomes (including 19 individuals from Benin that were CATT-wb-positive). For each ELISA test version, sensitivity and specificity were calculated for each PP and the PP with the highest Youden index was further used as a cut-off. The Youden index summarizes the sensitivity and specificity in a single value, which ranges between −1 and +1. The closer the Youden index is to +1, the better the test.

Binomial 95% confidence intervals (CI) of sensitivity and specificity were calculated. Sensitivities and specificities of the different ELISA test formats were compared using the McNemar chi-square test with Yates correction, with a 5% significance level.

Ethical considerations

All individuals providing serum or saliva gave their informed consent. Permission for the study was obtained from the national ethical committee of D. R. Congo, from the National Sleeping Sickness Control Programme of Benin and from the ITM ethical committee, reference number 03 07 1 413.

Results

Sensitivity, specificity and Youden index

Sensitivity, specificity and Youden index for the different ELISAs at different cut-offs are presented in Table 1.
Among the ELISAs on serum, the direct ELISA on serum dilution 1:150 showed the highest combination of sensitivity and specificity with a maximal Youden index of 0.979 at cut-off values between 34 and 44 PP. For the saliva tests, the highest Youden index (0.915) was observed with the indirect ELISA on saliva dilution 1:20 using a cut-off of six PP.

No significant difference in sensitivity or specificity could be detected between the different ELISA tests on saliva or between the ELISA tests on serum (Tables 2 and 3). The direct ELISA on serum was significantly more specific than the direct ELISAs on saliva or the indirect ELISA on saliva dilution 1:20 could be observed (P = 0.073). No significant difference in sensitivity between the ELISAs on serum and the ELISAs on saliva was observed (P ≥ 0.073). Compared with CATT, only the direct ELISA on saliva dilution 1:4 was significantly less sensitive (P = 0.041). However, all ELISA test formats on serum were significantly more specific than CATT on serum, and all ELISA test formats on serum and saliva were significantly more specific than the CATT-wb.

### Table 1: Sensitivity, specificity and Youden index of different test formats for trypanosome-specific antibody detection in blood, serum and saliva

<table>
<thead>
<tr>
<th>Sample</th>
<th>Test format</th>
<th>Sample dilution</th>
<th>Cut-off</th>
<th>Youden index</th>
<th>Percentage specificity (N = 128; CI)</th>
<th>Percentage sensitivity (N = 78; CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva</td>
<td>Direct ELISA</td>
<td>1:8</td>
<td>PP 2</td>
<td>0.881</td>
<td>94.5 (89.1–97.8)</td>
<td>93.6 (85.7–97.9)</td>
</tr>
<tr>
<td></td>
<td>Direct ELISA</td>
<td>1:4</td>
<td>PP 1</td>
<td>0.868</td>
<td>94.5 (89.1–97.8)</td>
<td>92.3 (84.0–97.1)</td>
</tr>
<tr>
<td></td>
<td>Indirect ELISA</td>
<td>1:40</td>
<td>PP 4</td>
<td>0.894</td>
<td>94.5 (89.1–97.8)</td>
<td>94.9 (87.4–98.6)</td>
</tr>
<tr>
<td></td>
<td>Indirect ELISA</td>
<td>1:20</td>
<td>PP 6</td>
<td>0.915</td>
<td>95.3 (90.1–98.3)</td>
<td>96.2 (89.2–99.2)</td>
</tr>
<tr>
<td>Serum</td>
<td>Direct ELISA</td>
<td>1:150</td>
<td>PP 34–44</td>
<td>0.979</td>
<td>99.2 (95.7–100.0)</td>
<td>98.7 (93.1–100.0)</td>
</tr>
<tr>
<td></td>
<td>Indirect ELISA</td>
<td>1:30 000</td>
<td>PP 15–17</td>
<td>0.951</td>
<td>97.7 (93.3–99.51)</td>
<td>97.4 (91.0–99.7)</td>
</tr>
<tr>
<td></td>
<td>Indirect ELISA</td>
<td>1:15 000</td>
<td>PP 15</td>
<td>0.959</td>
<td>98.4 (94.5–99.9)</td>
<td>97.4 (91.0–99.7)</td>
</tr>
<tr>
<td></td>
<td>CATT</td>
<td>1:4</td>
<td>1:4</td>
<td>0.883</td>
<td>88.3 (81.4–93.3)</td>
<td>100 (96.2–100.0)</td>
</tr>
<tr>
<td>Blood</td>
<td>CATT</td>
<td>Undiluted</td>
<td>Score ≥+</td>
<td>0.852</td>
<td>85.2 (77.2–90.8)</td>
<td>100 (96.2–100.0)</td>
</tr>
</tbody>
</table>

PP, percentage positivity; score ≥+, an agglutination in CATT of + or stronger compared with the reference card; ELISA, enzyme-linked immunosorbent assay; CATT, card agglutination test for trypanosomiasis; CI, confidence interval.

### Table 2: P-values of comparison of test sensitivities by McNemar chi-square test with Yates continuity correction

<table>
<thead>
<tr>
<th>Saliva</th>
<th>Serum</th>
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<tbody>
<tr>
<td>Direct ELISA</td>
<td>Direct ELISA</td>
</tr>
<tr>
<td>1:8</td>
<td>1:4</td>
</tr>
<tr>
<td>0.617</td>
<td>0.371</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Saliva</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct ELISA, 1:150</td>
<td>0.073</td>
</tr>
<tr>
<td>Indirect ELISA, 1:30 000</td>
<td>0.133</td>
</tr>
<tr>
<td>Indirect ELISA, 1:15 000</td>
<td>0.133</td>
</tr>
<tr>
<td>CATT, 1:4</td>
<td>0.133</td>
</tr>
</tbody>
</table>

*Bold indicate significant difference (P < 0.05).

NA, not applicable (χ² = 0); ELISA, enzyme-linked immunosorbent assay; CATT, card agglutination test for trypanosomiasis.
pre-dilution of the positive control in the saliva ELISAs, the antibody concentration in saliva was about 500 times lower than in serum.

Levey-Jennings charts
For all different ELISA test formats, the average OD of the positive control of the single plates fell between the limits of the overall average of all runs ±2 SDs. Levey-Jennings charts for the direct ELISA on serum and the indirect ELISA on saliva dilution 1:20 are illustrated in Figure 2.

Table 3  P-values of comparison of test specificities by McNemar chi-square test with Yates continuity correction

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<thead>
<tr>
<th></th>
<th>Saliva</th>
<th>Serum</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Direct ELISA</td>
<td>Indirect ELISA</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>1:4</td>
</tr>
<tr>
<td></td>
<td>Direct ELISA, 1:4</td>
<td>0.479</td>
</tr>
<tr>
<td></td>
<td>Indirect ELISA, 1:40</td>
<td>0.683</td>
</tr>
<tr>
<td></td>
<td>Indirect ELISA, 1:20</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>CATT, 1:4</td>
<td>NA</td>
</tr>
<tr>
<td>Blood</td>
<td>CATT, undiluted</td>
<td>$0.024^*$</td>
</tr>
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<td></td>
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*Significant difference ($P < 0.05$).
NA, not applicable ($\chi^2 = 0$); ELISA, enzyme-linked immunosorbent assay; CATT, card agglutination test for trypanosomiasis.

Figure 1  Relationship between serum and saliva trypanosome-specific antibody concentrations in 78 sleeping sickness patients.

Discussion
Based on the encouraging results of our pilot study (Lejon et al. 2003), we continued our research on detection of trypanosome-specific antibodies in saliva of sleeping sickness patients. The overall objective was to further optimize and evaluate ELISA/T. b. gambiense on saliva for non-invasive diagnosis of sleeping sickness.

In order to increase the analytical sensitivity, an indirect ELISA format was chosen in which the detection of specific antibodies in serum and saliva is enhanced with a secondary antibody directed against human IgG. An existing indirect ELISA, developed for trypanosome-specific antibody detection in serum and CSF, was fine-tuned for use on saliva. As in saliva, IgG concentrations in CSF are between 500 and 1000 times lower than in serum, and the sample dilution of CSF in this test format was 1:50. After cross-titration, two optimal saliva dilutions of 1:20 and 1:40 were retained (data not shown). The existing direct ELISAs for serum and saliva (Büscher et al. 1994; Lejon et al. 2003) were optimized, keeping the original serum dilution of 1:150 and adapting the saliva dilution to 1:4 and 1:8.

Once optimized, the direct and indirect ELISA/T. b. gambiense were tested on a collection of samples from sleeping sickness and negative controls. Contrary to the pilot study, all parasite negative controls used in the present study originated from sleeping sickness endemic areas. Due to the higher prevalence of other infections such as malaria, filariasis and schistosomiasis in these regions, more cross-reactions can be expected to occur in serological tests. Nevertheless, sensitivity and specificity of all test
formats were above 90%, using either serum or saliva. As observed previously, the antibody concentration was about 500 times lower in saliva than in serum. There was a linear relationship between serum and saliva antibody concentrations in the indirect ELISA. The saliva antibody concentration therefore reflects the concentration in blood, but the individual variations were high, as could be expected (McKie et al. 2002).

As we were dealing with four possible test formats for antibody detection in saliva (indirect ELISA with saliva dilutions 1:20 and 1:40 and direct ELISA with saliva dilutions 1:4 and 1:8) and three test formats for serum (indirect ELISA with serum dilutions 1:15 000 and 1:30 000 and direct ELISA with serum dilution 1:150), a choice for the best test version had to be made. Sensitivity and specificity were therefore compared, which should allow us to choose the test format with the highest diagnostic accuracy. For the ELISAs on saliva we could not observe a difference in sensitivity and specificity between the different test formats. Although both sensitivity and specificity were highest for the direct ELISA on serum, we could not observe a statistical difference with the indirect test formats on serum. Variability between the plates as evaluated in the Levey-Jennings graphs was lower with the direct ELISA than the indirect ELISAs on serum (data not shown). Based on the highest sensitivity and specificity, in combination with the best repeatability, the direct test format remains the best choice for testing serum in ELISA.

As our goal was to replace the classical invasive tests on serum or blood by a non-invasive test on saliva, we wondered if the saliva tests would perform worse than serum tests. While we observed no statistical difference in sensitivity, a significantly lower specificity of both direct ELISA formats on saliva compared with the direct serum ELISA was observed. Our direct ELISAs on saliva therefore seem slightly inferior to the direct ELISA on serum. For the indirect ELISAs on saliva, we did not observe a difference in sensitivity with the direct serum ELISA. Only the specificity of the indirect ELISA on saliva dilution 1:20 was not significantly lower than the specificity of the direct ELISA on serum. The best test format for saliva testing therefore seems the indirect ELISA on sample dilution 1:20. This is the only test version for which we could not observe any difference in test performance with the direct serum ELISA.

Based on our results, we can therefore replace the direct ELISA on serum by an indirect ELISA on saliva dilution 1:20. With this test, similar sensitivities can be expected (96.34%, CI: 89.7–99.2% for saliva compared with 98.77%, CI: 93.3–99.97% for serum). In practice, the specificity might be slightly lower for the saliva ELISA (95.45%, CI: 90.4–98.3) than for the serum ELISA (98.77%, CI: 93.3–99.97). The replacement of an invasive serum ELISA by a non-invasive ELISA on saliva dilution 1:20 might therefore result in detection of more false-positives, thus selection of more non-infected persons for parasitological examination.

As in the field, screening of the population is carried out by CATT, not by ELISA, one should rather compare the performance of the indirect ELISA on saliva dilution 1:20 with CATT. No difference in sensitivity was observed, but the specificity of the indirect ELISA on saliva dilution 1:20 was higher than in CATT, especially CATT-wb. Therefore, conclude that we can replace both the direct ELISA on serum, as well as the CATT-wb by an ELISA on saliva for non-invasive diagnosis of sleeping sickness without loosing test performance. As demonstrated previously, the ease and safety of saliva collection and its social acceptability make saliva testing well suited for such epidemiological purposes, screening of large populations and repeated samplings.

In order to fully exploit the advantages of non-invasive saliva testing, the format should be a point-of-care test that is applicable for mass screening of the population at risk in rural Africa. An ELISA test does not fulfil these criteria. The execution of an ELISA requires large volumes of pure water, requires pipettes, and most secondary antibodies and conjugates are not stable at ambient temperatures. Moreover, the test takes a few hours. Unfortunately, existing field tests such as CATT or LATEX/T. b. gambiense are of no use with saliva due to their insufficient analytical sensitivity and the occurrence of unspecific agglutination reactions (Lejon et al. 2003). The main challenge therefore remains the development of a saliva test that can be performed under field conditions. Possible test formats include lateral flow tests or gel agglutination tests. The recent approval by the American Federal Drug Administration of a lateral flow test for HIV-specific antibody detection in saliva which can be performed in 20 min, demonstrates the feasibility of fast and simple test formats that are applicable on saliva (http://www.fda.gov/bbs/topics/news/2004/NEW01042.html).

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References


Detection of specific antibodies of trypanosome in saliva - Toward a non-invasive serological diagnosis of sleeping sickness

OBJECTIVE La détection d’anticorps spécifiques de trypanosome dans la salive est une technique réalisable et si elle est validée cliniquement, elle pourrait devenir une option attrayante pour le diagnostic sérologique non-invasif de la maladie du sommeil. Nous avons voulu optimiser le format d’un test ELISA basé sur le système de détection d’anticorps.

MÉTHODES Divers formats d’ELISA pour la détection d’anticorps dans le sérum et la salive ont été développés et standardisés. Les échantillons de salive et de sérum ont été collectés chez 78 patients et 128 contrôle endémiques. La sensibilité et la spécificité des ELISA sur salive, sur sérum et du test de l’agglutination sur carte pour la trypanosomiase (test CATT) ont été évaluées.

RÉSULTATS Tous les formats d’ELISA ont démontré une sensibilité et une spécificité supérieures à 90%. Les ELISA sur salive ont démontré une performance de test similaire à celle des ELISA sur sérum et à celle du test CATT sur sang total ou sur sérum.

CONCLUSIONS Cette étude confirme la détection possible d’anticorps spécifiques de trypanosome dans la salive.

MOTS CLÉS trypanosomiase humaine Africaine, détection d’anticorps, salive, sérum diagnostic, Trypanosoma brucei gambiense

Detection en saliva de anticuerpos específicos para tripanosoma: hacia un diagnóstico serológico no invasivo para la enfermedad del sueño

OBJETIVO La detección de anticuerpos específicos para tripanosoma en saliva es técnicamente posible, y si se valida desde un punto de vista clínico, podría convertirse en una opción atractiva para el diagnóstico no invasivo de la enfermedad del sueño. Buscábamos optimizar el formato de la prueba en un sistema de detección de anticuerpos basado en un ELISA.

MÉTODOS Se desarrollaron y estandarizaron diferentes formatos de ELISA para la detección de anticuerpos en suero y saliva. Se recolectaron muestras de suero y saliva de 78 pacientes y 128 controles provenientes de zonas endémicas, y se evaluó la sensibilidad y la especificidad del ELISA para saliva, del ELISA para suero y de las pruebas para tripanosomiasis de aglutinación en tarjetas (CATT).

RESULTADOS Todos los formatos de ELISA mostraron una sensibilidad y especificidad por encima del 90%. Los ELISA para saliva tuvieron un desempeño similar al ELISA para suero y el CATT con sangre y con suero.

CONCLUSIONES Este estudio confirma el potencial de la detección en saliva de anticuerpos específicos para tripanosoma.

PALABRAS CLAVE tripanosomiasis humana Africana, detección de anticuerpos, saliva, suero, diagnóstico, Trypanosoma brucei gambiense