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# A new format of the CATT test for the detection of Human African Trypanosomiasis, designed for use in peripheral health facilities

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**Summary**

OBJECTIVES To test the reproducibility and thermostability of a new format of the Card-Agglutination Test for Trypanosomiasis (CATT) test for Human African Trypanosomiasis (HAT), designed for use at primary health care facility level in endemic countries.

METHODS A population of 4217 from highly endemic villages was screened using the existing format of the CATT test (CATT-R250) on whole blood. All those testing positive (220) and a random sample of negatives (555) were retested in the field with the new format (CATT-D10). Inter-format reproducibility was assessed by calculating kappa. All samples testing positive on whole blood with either method were further evaluated in Belgium by CATT titration of serum by two observers, using both old and new format. CATT-D10 test kits were incubated under four temperature regimens (4, 37, 45 °C and fluctuating) with regular assessments of reactivity over 18 months.

RESULTS Inter-format reproducibility of CATT-D10 *vs*. CATT-R250 on whole blood performed by laboratory technicians in the field was excellent with kappa values of 0.83–0.89. Both inter- and intra-format reproducibility assessed by CATT titration were excellent, with 96.5–100% of all differences observed falling within the limits of ±1 titration step. After 18 months, reactivity of test kits incubated under all four temperature regimens was still well above the minimum threshold considered acceptable.

CONCLUSION The CATT-D10 is thermostable and can be used interchangeably with the old format of the CATT test. It is highly suitable for use in peripheral health facilities in HAT-endemic countries.

**keywords** trypanosomiasis, *Trypanosoma brucei gambiense*, agglutination tests, mass screening, primary health care, communicable disease control

## Background

The Card-Agglutination Test for Trypanosomiasis (CATT) was developed in the late 1970s and has been hailed as a breakthrough in the diagnosis of *Trypanosoma brucei gambiense* sleeping sickness (Magnus *et al.* 1978). The CATT is a fast and simple direct agglutination assay for detection of *T. b. gambiense*-specific antibodies in the blood, plasma or serum of patients with Human African Trypanosomiasis (HAT). In mass screening programmes for the disease, the CATT is used as a screening test, followed by parasitological confirmation (Chappuis *et al.* 2005; Lutumba *et al.* 2005).

Over the past decade, HAT prevalence has fallen in most endemic countries. This is also the case in the Democratic Republic of Congo (DRC), which still accounts for about 60% of all cases reported worldwide (WHO 2006). This reduced prevalence has led to calls for integration of HAT control into the general primary health care (PHC) system. Designed for use in mass screening programmes, the current CATT test is produced in vials of 50 tests units which cannot be kept once the vial has been reconstituted.

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This would lead to waste of CATT antigen in most PHC facilities in endemic areas, as they see fewer patients daily. Moreover, the CATT test cannot be kept at ambient temperatures for long, precluding its use in peripheral health facilities without a cold chain.

To overcome these limitations, a new format of the CATT test was developed by the Institute of Tropical Medicine in Antwerp, Belgium (ITMA). The new format is based on a thermostable lyophilisation medium and produced in 10-unit vials (CATT-D10). Initial testing in the laboratory was successful (data not shown). This paper reports the field evaluation of the new CATT-D10 in the DRC, along with thermostability tests at the laboratory in Antwerp.

## Materials and methods

### Patients and samples

In December 2008, 4217 persons living in six highly endemic villages in the province of East Kasaï in the DRC were enrolled for HAT screening by a mobile unit using the CATT test on whole blood. Two hundred and twenty subjects tested positive, all of whom were enrolled as 'cases'. Out of the remaining 3997 who tested negative, 555 randomly selected persons were enrolled as controls. For each case and each control, four additional capillary tubes of whole blood were collected. These samples of cases and controls were tested on the spot by four laboratory technicians who did not know the previous results; two technicians were using the old format and two were using the new format of the CATT test. All four laboratory technicians were experienced staff of the national HAT control program (PNLTHA) with ample training and experience in performing the CATT test. Venous blood was collected from all subjects testing positive and serum samples were prepared. These serum samples were sent to the ITMA, Belgium, on dry-ice, for CATT titration using both the old (CATT-R250) and the new format (CATT-D10).

### Reagents

The antigen was prepared in the Applied Technology and Production Unit of ITMA. The CATT antigen consists of a di-ethylaminoethylcellulose-column purified, formaldehyde-fixed and Coomassie-blue-stained bloodstream-form of *T. b. gambiense* variable antigen type LiTat 1.3. The antigen suspension was divided into two equal volumes. One volume was processed with the standard CATT lyophilisation medium, divided over separate vials each containing 1.05 ml, and freeze dried (R250). For the other volume, a different lyophilisation medium was used: the volume was divided over separate vials each containing 0.375 ml, and freeze dried (D10). The protocol used for freeze drying the R250 and D10 antigens was identical. Prior to use, the antigens were re-suspended with CATT-buffer, 2.5 ml for R250 and 0.75 ml for D10 which results in 50 doses and 10 doses per vial, respectively. After lyophilisation, samples of both antigens were tested against a panel of well-characterised reference sera.

The CATT-buffer is composed of phosphate-buffered saline (pH 7.2) supplemented with 0.1% sodium azide.

A diluted, freeze-dried positive goat serum with a CATT titre of 1/8 was used as positive control. As negative control, a freeze-dried bovine serum albumin suspension was used. In the D10 kits, the control sera were freeze dried with the same modified lyophilisation medium as used with the antigen.

Reagents and accessories for use in DRC were sent to the University of Kinshasa in the DRC. The R250 was sent under cold chain conditions, whereas the D10 was sent at ambient temperature.

### CATT test

Results were read after orbital shaking for 5 min at 0.05 *g*. In the field agglutination patterns for CATT on whole blood were scored as '−', '±', '+', '++' or '+++', depending on the intensity of the agglutination. Results of '±' or above were considered positive. At the ITMA laboratory, agglutination patterns for CATT dilution were scored from 0 (absence of agglutination) to 3 (very strong agglutination), a score of 0.5 or above being considered positive.

In the DRC, the CATT test on whole blood samples of 775 persons (220 labelled positive and 555 labelled negative by the mobile unit) was repeated in the field by four laboratory technicians, two using the old format CATT-R250 and two using the new format, CATT-D10. All tests were carried out blindly without prior knowledge of the results of the mobile unit. All sera from persons labelled CATT positive on whole blood, either by the mobile unit using CATT-R250 or by any of the four readers during the second reading, using CATT-R250 or CATT-D10, were re-examined by CATT titration at ITMA in Antwerp.

At ITMA, two readers performed CATT titration using the old and the new format. This was done on five serial dilutions of serum, starting with a 1:4 dilution and ending with a dilution of 1:64. For each dilution step, the reader assigns a score from 0 to 3 as described earlier. The highest dilution with a score of 0.5 or above is considered the end titre.

One of the readers at ITMA retested the same samples with both formats to assess intra-reader variability. The tests were done on different days in a blinded manner; the readers had no prior knowledge of earlier results for the same sample with either test format or by either reader.

### Thermostability

Thermostability was tested by incubating the CATT-D10 reagent under four temperature regimens. One batch was incubated at temperatures alternating initially weekly, later monthly, between 4, 20 and 37 °C. Three other batches were incubated at constant temperatures of 4, 37 and 45 °C. Reactivity was assessed against standard reference sera used for quality control of the old format of the CATT test, once a month during the first 6 months and at six monthly intervals thereafter.

For quantifying reactivity of the CATT, we used the criteria that have been in use for the routine quality assurance of the CATT test at ITMA since 1988; the main criterion being the average end titre against a well-characterised panel of 10 reference sera. Values obtained for a 1988 batch of CATT are considered the minimum acceptable level; reactivity for any new batch needs to be at least as good. For a batch to pass the quality assurance check, the average end titre needs to be 1.7 or above.

### Data analysis

For CATT on whole blood, agreement was assessed on a binary scale using Cohen's kappa coefficient (Cohen 1960). Kappa coefficients were interpreted following Landis and Koch (1977): 1.00–0.81 excellent, 0.80–0.61 good, 0.60–0.41 moderate, 0.40–0.21 weak and 0.20–0.00 negligible agreement.

For CATT titration, we assessed the agreement by calculating proportions of differences in end titres. For this purpose, end titres obtained as described earlier in the 'test execution' section were recoded to a linear scale. Systematic error was estimated by the mean difference of all paired titre differences $x_i - y_i$ and tested by Wilcoxon signed-rank tests.

All data were entered in a Microsoft Excel format and analysed with Stata10 (Stata Corp., College Station TX, USA).

### Ethical aspects

The protocol for this study was approved by the Institutional Review Board of the ITMA in Belgium and the Ethics Committee of the Public Health School of Kinshasa University in DRC. Informed consent was asked from each person before inclusion in the study. Patients with HAT identified during the study were treated in accordance with the standard protocols of the PNLTHA of the DRC.

## Results

### Comparison of results on whole blood

While repeating the CATT test on whole blood in the field, seven additional CATT-positives were identified among the controls between the four laboratory technicians. When comparing the results of the mobile unit (reader 1) with those of the laboratory technicians (readers 2 and 3), all using the old R-250 format, kappa values of 0.78 and 0.79 were obtained. Agreement between the two laboratory technicians (readers 2 and 3) using the same format was better, with a kappa value of 0.84. There were no major changes in kappa values when the results of the mobile unit (reader 1) using the old R250 format were compared with the results obtained by two other laboratory technicians (readers 4 and 5) using the new D10 format; kappa values obtained were 0.74 and 0.77. The kappa value for agreement between two laboratory technicians both using the D10 format (reader 4–5) was 0.83, comparable to the agreement between the two laboratory technicians using the R250 format (readers 2 and 3). There were four possible combinations between the two formats used by laboratory technicians, kappa values obtained ranged from 0.81 to 0.89 (Table 1).

### Comparison of CATT titration results

Of 227 serum samples prepared, one was lost during transport. The remaining 226 serum samples were tested

**Table 1** Agreement between readers and between formats for CATT on whole blood in the field

| Comparison CATT reading | Kappa |
|---|---|
| R250: reader 1 *vs*.reader 2 | 0.78 |
| R250: reader 1 *vs*. reader 3 | 0.79 |
| R250: reader 2 *vs*. reader 3 | 0.84 |
| D10: reader 4 *vs*. reader 5 | 0.83 |
| R250: reader 1 *vs*. D10 reader 4 | 0.74 |
| R250: reader1 *vs*. D10 reader 5 | 0.77 |
| R250: reader 2 *vs*. D10 reader 4 | 0.83 |
| R250: reader 2 *vs*. D10 reader 5 | 0.81 |
| R250: reader 3 *vs*. D10 reader 4 | 0.89 |
| R250: reader 3 *vs*. D10 reader 5 | 0.83 |

Reader 1, mobile team technician using CATT-R250; Readers 2 and 3, research team laboratory technicians using CATT-R250; Readers 4 and 5, research team laboratory technicians using D10; CATT, Card-Agglutination Test for Trypanosomiasis.

**Table 2** Differences in Card-Agglutination Test for Trypanosomiasis titration results between readers and between formats for 226 samples tested at ITMA

| | Proportion of difference in titration steps | | | | | | |
|---|---|---|---|---|---|---|---|
| Comparison | −2 | −1 | 0 | 1 | 2 | Mean difference (*P*-value*) | ≤±1 (95% CI) |
| R250, inter-reader readers 1 and 2, ITMA | 0% | 11.5% | 65.9% | 22.1% | 0.4% | 0.115 (0.0042) | 99.6% (97.2–100%) |
| D10, inter-reader readers 1 and 2, ITMA | 0% | 23.0% | 68.1% | 8.8% | 0% | −0.142 (0.0002) | 100% (97.9–100%) |
| R250, intra-reader reader 1, ITMA | 0% | 18.1% | 71.7% | 10.2% | 0% | −0.08 (0.0244) | 100% (97.9–100%) |
| D10, Intra-reader reader 1, ITMA | 0% | 8.0% | 76.1% | 15.5% | 0.4% | 0.084 (0.0138) | 99.6% (97.2–100%) |
| R250 *vs.* D10, inter-format reader 1, ITMA | 3.5% | 42.5% | 52.2% | 1.8% | 0 | 0.478 (<0.00005) | 96.5% (92.9–98.3%) |
| R250 *vs.* D10, inter-format reader 2, ITMA | 1.3% | 25.2% | 68.1% | 4.9% | 0.4% | 0.221 (<0.00005) | 98.2% (95.2–99.4%) |

ITMA, Institute of Tropical Medicine in Antwerp.
*Based on Wilcoxon signed-rank test.

by CATT titration at ITMA as described earlier. Of all differences between the two ITMA readers for R250, 99.6% (95% CI 97.2–100%) fall within the limits of ±1 titration step; for the D10, this even was 100% (95% CI 97.9–100%). Regarding the same observer with two formats, the proportion of differences falling within the boundaries of ±1 titration step was 96.5% (95% CI 92.9–98.3%) and 98.2% (95% CI 95.2–99.4%) for readers 1 and 2, respectively. Titres obtained with the R250 format were on average higher than those obtained with D10, 0.478 and 0.221 titres respectively for ITMA reader 1 and ITMA reader 2 ($P < 0.00005$ in both cases; Table 2).

### Thermostability tests

The CATT-D10 test kits and the positive control sera retained their reactivity under all four temperature regimens. After an initial drop in the first month, reactivity remained acceptable and fairly stable with minor fluctuations over 18 months. Data for the 45 °C and fluctuating temperatures regimen are presented in Figure 1 below.

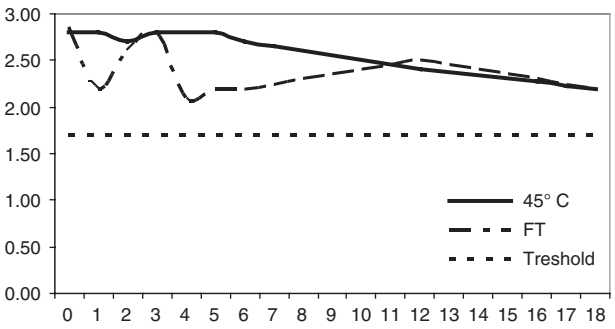


**Figure 1** Average end titres after incubation at fluctuating temperatures or 45 °C.

## Discussion

The CATT-D10 is specifically designed for use at peripheral health centre level. This study shows that under field conditions, the CATT-D10 on whole blood performs as well as the CATT-R250. Inter-reader agreement for the CATT-D10 between laboratory technicians in the field is excellent. Even combined inter-reader and inter-format agreement for laboratory technicians in the field is excellent.

Further testing at the laboratory in Antwerp showed that results for CATT titration using the CATT-D10 are also highly reproducible. Both inter- and intra-reader reproducibility for the CATT-D10 are very high, with >99% of all differences observed falling within the range of ±1 titration step. When comparing both formats, we did notice that titres obtained with CATT-R250 are slightly but significantly higher than those obtained with the new format. But these differences are so small that they do not have practical implications. In >96% of cases, the differences between the two formats are within the acceptable limits of ±1 titration step, a criterion that is generally accepted for this type of direct agglutination test readings when read on a quantitative scale.

Results of thermostability testing were excellent. Whereas earlier tests (R. Baelmans and D. Jacquet, unpublished observation) showed that the CATT-R250

loses reactivity within 1 month of exposure to high temperatures, for the CATT-D10, there is no loss in reactivity that would have any practical significance even after exposure to very high or alternating temperatures over 18 months. This is very important for using the CATT test at PHC facilities without a cold chain.

The CATT-D10 is produced in vials of 10 dosages, whereas CATT-R250 vials contain 50 doses. The downside of the smaller unit size is an increase in shipping volume and production costs per test unit. For mass screening programmes which use the CATT test in large quantities and face fewer constraints in terms of cold chain availability, CATT-R250 will remain the format of choice. But in peripheral health centres, the smaller unit size of CATT-D10 will reduce waste of CATT antigen; PHC facilities in the DRC are typically attended by fewer than 20 persons a day (Wembonyama *et al.* 2007).

## Conclusion

The CATT-D10 performs as well as the CATT-R250, and both tests can be used interchangeably. Because of its thermostability and because of its smaller unit size, the CATT-D10 permits using a CATT test at PHC facilities.

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