Control of chronic infectious diseases in low resource settings
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Chapter 8. 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
We evaluated a new format of the CATT test for Human African Trypanosomiasis (HAT), designed for use at PHC facility level in endemic countries. We tested reproducibility and thermo stability.

Methods
A population of 4,217 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 re-tested 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 2 different observers, using both old and new format. CATT-D10 test kits were incubated under 4 different temperature regimens (4ºC, 37ºC, 45 ºC and fluctuating) with regular assessments of reactivity over an 18 month period.

Results
Inter format reproducibility of CATT-D10 vs. CATT-R250 on whole blood performed by lab 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 plus or minus 1 titration step. After 18 months, reactivity of test kits incubated under all 4 different temperature regimens was still well above the minimum threshold considered acceptable.

Conclusion
The CATT-D10 is thermo stable 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.
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Background
The Card Agglutination Test for Trypanosomiasis (CATT) was developed in the late 1970's and has been hailed as a breakthrough in the diagnosis of T. b. gambiense sleeping sickness. 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 human African trypanosomiasis (HAT) patients. In mass screening programs for the disease, the CATT is used as a screening test, followed by parasitological confirmation tests.

Over the past decade HAT prevalence has decreased 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. 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 programs, the current CATT-test is produced in vials of 50 tests units which cannot be kept once the vial has been reconstituted. This would lead to wastage of CATT antigen in most PHC facilities in endemic areas, as they see much less patients on a daily basis. Moreover the CATT test cannot be kept at ambient temperatures for longer periods, precluding its use in peripheral health facilities that lack a functional cold chain.

To overcome these limitations, a new format of the CATT test has been developed by the Institute of Tropical Medicine in Antwerp, Belgium (ITMA). The new format is based on a thermo stable lyophilisation medium and is 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 thermo stability tests at the laboratory in Antwerp.

Materials and Methods

Patients and Samples
During December 2008, 4217 persons living in 6 highly endemic villages in the province of East Kasai in the DRC were enrolled for HAT screening by a mobile unit using the CATT test on whole blood. Two hundred 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, 4 additional capillary tubes of whole blood were collected. These samples of cases and controls were tested on the spot by 4 different laboratory technicians who did not know the previous results; 2 technicians were using the old format and 2 were using the new format of the CATT test. All 4 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
Antigen
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 \textit{T.b. gambiense} variable antigen type LiTat 1.3. The antigen suspension was divided into 2 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, it 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 respectively 50 doses and 10 doses per vial. After lyophilisation, samples of both antigens were tested against a panel of well-characterized reference sera.

**Buffer and Diluents**
The CATT-buffer is composed of Phosphate Buffered Saline (PBS pH 7.2) supplemented with 0.1% sodium azide.

**Control sera**
A diluted, freeze dried positive goat serum with a CATT titre of 1/8 is used as positive control. As negative control a freeze dried Bovine Serum Albumin suspension is used. In the D10 kits the control sera have been freeze dried with the same modified lyophilisation medium as used with the antigen.

**Shipping conditions**
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.

**TEST EXECTION**

**CATT test**
Results were read after orbital shaking for 5 minutes at 60 rpm. In the field agglutination patterns for CATT on whole blood were scored as ‘-’, ‘±’, ‘+’, ‘++’ or ‘+++’, depending on the intensity of agglutination observed. 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 4 laboratory technicians, 2 using the old format CATT-R250 and 2 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 4 readers during the second reading, using CATT-R250 or CATT-D10, were re-examined by CATT titration at ITMA in Antwerp.

At ITMA 2 readers performed CATT titration using the old as well as the new format. This was done on 5 serial dilutions of serum, starting from a 1:4 dilution until a maximum dilution of 1:64. For each dilution step, the reader assigns a score from 0-3 as described above. The highest dilution with a score of 0.5 or above is considered the end titre. One of the readers at ITMA tested the same samples once again with
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Comparing the results of 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.

Thermo Stability
Thermo stability was tested by incubating the CATT-D10 reagent under 4 different temperature regimens. One batch was incubated at temperatures alternating initially on a weekly basis, later on a monthly basis, between 4°C, 20°C and 37°C. Three other batches were incubated at constant temperatures of 4°C, 37°C 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 in 6-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 characterized 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. Kappa coefficients were interpreted following Landis and Koch: 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 above 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 as well as 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. HAT patients identified during the study were treated in accordance with the standard protocols of the national HAT control program (PNLTHA) of the DRC.

Results
Comparison of Results on Whole Blood
While repeating the CATT test on whole blood in the field, 7 additional CATT-positives were identified among the controls between the 4 laboratory technicians. When comparing the results of the mobile unit (reader 1) with those of the laboratory technicians (reader 2-3), all using the old R-250 format, kappa values of 0.78 and 0.79 were obtained. Agreement between the 2 laboratory technicians (reader 2-3) 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 2 other laboratory technicians (reader 4-5) using the new D10 format; kappa values obtained were 0.74 and 0.77. The kappa value for agreement between 2 laboratory technicians both using the D10 format (reader 4-5) was 0.83, comparable to the agreement between the 2 laboratory technicians using the R250 format (reader 2-3). There were 4 possible combinations between the 2 formats used by laboratory technicians, kappa values obtained ranged from 0.81 to 0.89 (table 1).

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

<table>
<thead>
<tr>
<th>COMPARISON CATT READING</th>
<th>KAPPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>R250 : reader 1 vs.reader 2</td>
<td>0.78</td>
</tr>
<tr>
<td>R250: reader 1 vs. reader 3</td>
<td>0.79</td>
</tr>
<tr>
<td>R250: reader 2 vs. reader 3</td>
<td>0.84</td>
</tr>
<tr>
<td>D10: reader 4 vs. reader 5</td>
<td>0.83</td>
</tr>
<tr>
<td>R250 reader 1 vs. D10 reader 4</td>
<td>0.74</td>
</tr>
<tr>
<td>R250 reader1 vs. D10 reader 5</td>
<td>0.77</td>
</tr>
<tr>
<td>R250 reader 2 vs. D10 reader 4</td>
<td>0.83</td>
</tr>
<tr>
<td>R250 reader 2 vs. D10 reader 5</td>
<td>0.81</td>
</tr>
<tr>
<td>R250 reader 3 vs. D10 reader 4</td>
<td>0.89</td>
</tr>
<tr>
<td>R250 reader 3 vs. D10 reader 5</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Reader 1: mobile team technician using CATT-R250, Reader 2-3: research team lab technicians using CATT-R250, Reader 4-5 research team lab technicians using D10.

**Comparison of CATT titration results**

Out of 227 serum samples prepared, 1 sample was lost during transport. The remaining 226 serum samples were tested by CATT titration at ITMA as described above. Of all observed differences between the 2 ITMA readers for R250, 99.6% (95% CI 97.2-100%) fall within the limits of plus or minus one titration step; for the D10 this is even 100% (95% CI 97.9-100%). Comparing the same observer with 2 different formats, the proportion of differences that fall within the boundaries of plus or minus 1 titration step are 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).
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Table 2: Observed differences in CATT titration results between readers and between formats for 226 samples tested at ITMA

<table>
<thead>
<tr>
<th>COMPARISON</th>
<th>PROPORTION OF DIFFERENCE IN TITRATION STEPS</th>
<th>Mean difference (p-value*)</th>
<th>≤±1 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-2</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>R250, Inter reader readers 1 &amp; 2, ITMA</td>
<td>0%</td>
<td>11.5%</td>
<td>65.9%</td>
</tr>
<tr>
<td>D10, Inter reader readers 1 &amp; 2, ITMA</td>
<td>0%</td>
<td>23.0%</td>
<td>68.1%</td>
</tr>
<tr>
<td>R250, Intra reader reader 1 ITMA</td>
<td>0%</td>
<td>18.1%</td>
<td>71.7%</td>
</tr>
<tr>
<td>D10, Intra reader reader 1 ITMA</td>
<td>0%</td>
<td>8.0%</td>
<td>76.1%</td>
</tr>
<tr>
<td>R250 vs. D10, inter format reader 1 ITMA</td>
<td>3.5%</td>
<td>42.5%</td>
<td>52.2%</td>
</tr>
<tr>
<td>R250 vs. D10, inter format reader 2 ITMA</td>
<td>1.3%</td>
<td>25.2%</td>
<td>68.1%</td>
</tr>
</tbody>
</table>

* based on Wilcoxon signed-rank test

**Thermo Stability Tests**

The CATT-D10 test kits as well as the positive control sera retained their reactivity under all 4 temperature regimens tested. After an initial drop in the first month, reactivity remained acceptable and fairly stable with minor fluctuations over an 18 months period. 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 (FT) or 45°C](image)
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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 lab technicians in the field is excellent. Even combined inter-reader and inter-format agreement for lab 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 over 99% of all differences observed falling within the range of plus or minus 1 titration step. When comparing 2 formats we did notice that titres obtained with CATT-R250 are slightly but significantly higher than those obtained with the new format. These differences are however so small that they do not have practical implications. In over 96% of cases the differences observed between the 2 formats are within the acceptable limits of plus or minus 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 thermo stability testing are excellent. Whereas earlier tests (unpublished) showed that the CATT-R250 looses 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 a period of 18 months. This is of major importance when using the CATT test at PHC facilities that do not have a functional cold chain.

The CATT-D10 is produced in vials of 10 dosages, whereas CATT-R250 vials contain 50 dosages. The downside of the smaller unit size is an increase in shipping volume and production costs per test unit. For mass screening programs 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. However when used in peripheral health centres, the smaller unit size of CATT-D10 is a major advantage as it will reduce wastage of CATT antigen; PHC facilities in the DRC are typically attended by less than 20 persons a day.7

Conclusion

The CATT-D10 performs as well as the CATT-R250 and both tests can be used interchangeably. Because of its thermo stability and because of its smaller unit size, the CATT-D10 represents a break through which makes it possible to implement the CATT test at PHC facility level.
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References


