ANIMAL TRYPANOSOMOSIS: DIAGNOSIS AND EPIDEMIOLOGY

Results of a FAO/IAEA Co-ordinated Research Programme on the use of immunoassay methods for improved diagnosis of trypanosomosis and monitoring tsetse and trypanosomosis control programmes

GRAPHICAL CONTROL AND EVALUATION OF THE OPERATIONAL PERFORMANCE OF ELISA METHOD FOR DETECTION OF TRYPANOSOMAL ANTIBODIES

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Abstract

GRAPHICAL CONTROL AND EVALUATION OF THE OPERATIONAL PERFORMANCE OF ELISA METHOD FOR DETECTION OF TRYPANOSOMAL ANTIBODIES

The performance of four indirect trypanosomal antibody detection enzyme-linked immunosorbent assays (I-TAB ELISA's), exploiting native and denatured antigens of *Trypanosoma congolense* (*T.c.*AGn, *T.c.*AGd) and *T. vivax* (*T.v.*AGn, *T.v.*AGd), has been evaluated in fifteen laboratories from Africa and Europe. Standardised internal quality control samples (IQCs) were used as indicators and data plotted on charts to monitor and control the ELISA's at individual laboratories. Based on the overall data, dispersion of true values from the population data range was estimated plotting the location and deviation of raw and normalised absorbance values of the IQCs. Binding ratios were calculated to estimate the assay proficiency with respect to the accuracy of assessing that the IQC samples tested positive or negative in the test proper. The frequency distribution of coefficients of variation < 10 % of IQCs was monitored. The use of this standardised and transparent IQC data charting provides a useful quality control tool for the evaluation of the performance of the ELISA's used for trypanosomosis serology. The method provides a measure of confidence in estimating the proficiency of national laboratories with respect to reported ELISA results on disease occurrence.

Moreover, data were compiled of the inter-laboratory ELISA performance by means of summary data charts with reference to the performance criteria described. The data were also analysed using modified Youden plots. The analysis demonstrated similar laboratory proficiency for the I-TAB ELISA (*T.c.*AGn) (four of five laboratories), I-TAB ELISA (*T.c.*AGd) (eleven of fourteen laboratories), I-TAB ELISA (*T.v.*AGn) (three of five laboratories), and I-TAB ELISA (*T.v.*AGd) (eleven of fifteen laboratories). The impact of data variation between test and specified values as a sole decision criterion for acceptance or rejection of test plates is discussed.

1. INTRODUCTION

Work on serological methods has demonstrated that the enzyme-linked immunosorbent assay (ELISA) is the most suitable method for complementary use with traditional parasitological techniques to aid control and diagnosis of trypanosomosis in animal livestock caused by *Trypanosoma congolense*, *T. vivax* and *T. brucei* [1]. Indirect ELISA's have been evaluated for antibody detection in serum samples using trypanosomal crude antigen preparations or purified antigen fractions originating from rodents or cell culture conditions [2, 3, 4, 5]. For the detection of circulating trypanosomal antigens in serum samples, direct sandwich assays were developed exploiting monoclonal antibodies [6]. However, these efforts have not led to the distribution of a robust, sustainable and internationally recognized ELISA method. For international trade, methods for direct examination and parasite concentration rather than ELISA are still the prescribed tests for examination of tsetse-borne trypanosomosis by the Office International des Epizooties [7], although it is demonstrated that they provide low sensitivity with a high specificity.

Recently, four indirect ELISA's have been developed, [8, 9, 10]. In addition, a standardised and transparent control system monitoring the operational performance of the ELISA within specified limits was developed for implementation as routine application in diagnostic laboratories in the tropics.

The objective of this study was to obtain and present data on the quality control of four ELISA's for detecting antibodies against trypanosomosis through the use of charting methods. Such methods ensure the constant control and monitoring of the operational performance of ELISA's with

respect to the tentative control data ranges determined under conditions at the FAO/IAEA Agriculture and Biotechnology Laboratory, during the assay development stages. The data were processed using Shewhart-like charts [11], and used data from standardised internal quality control samples [12] referred to as ELISA performance indicator data. The methods gave immediate visual monitoring and helped in controlling the operational performance from plate to plate and day to day. The overall operational performance of the assays was compared from fifteen laboratories in Africa and Europe. The results were analysed graphically using summary data charts and modified Youden plots [13, 14].

2. MATERIALS AND METHODS

2.1. Laboratories

The operational performance of the ELISA's was monitored in fifteen laboratories in Austria and Belgium, Burkina Faso, Cameroon, Cote d'Ivoire, Ghana, Kenya, Mali, Nigeria, Sudan, Tanzania, Uganda, Zambia, Zanzibar and Zimbabwe.

2.2. ELISA reagents and shipment

Four indirect trypanosomosis antibody (I-TAB) ELISA systems were evaluated. Briefly, they exploited native (AGn) or detergent-heat treated antigen preparations (AGd): two *T. congolense* (*T.c.*) AG-based indirect ELISA's (I-TAB ELISA (*T.c.*AGn) and I-TAB ELISA (*T.c.*AGd)), as well as two *T. vivax* (*T.v.*) AG-based indirect ELISA's (I-TAB ELISA (*T.v.*AGn) and I-TAB ELISA (*T.v.*AGd)) [9]. The antigen-precoated ELISA plates were sealed, packed in plastic bags with silica gel desiccant packets (Sigma, USA), and stored at +37°C until shipment in the original cardboard boxes of the ELISA plate manufacturer by air freight without special conditions. The plates were stored at room temperature in counterpart laboratories until used. The frozen biological reagents (control sera and conjugated antibody) were dispatched in vacuum flasks and kept at -20°C until used.

2.3. ELISA procedure

The ELISA's were performed according to the corresponding standardized FAO/IAEA bench protocols (prototype version 1.0, November 1998). The assay procedure included the testing of four internal quality control (IQC) samples in four replicates: a defined strong positive (C++), a moderate positive (C+), a negative serum sample (C-), and serum diluent buffer as a conjugate control (Cc) as described elsewhere [12]. The IQCs were used as operational performance indicators of the ELISA method.

2.4. Tentative internal quality control limits

As part of the assay standardisation procedure at the FAO/IAEA Agriculture and Biotechnology Laboratory, preliminary internal quality control limits were established based on theconsensus from a Joint FAO/IAEA Meeting of Consultants convened in Vienna in January 1992. Replicates of each IQC (n=24) were repeatedly tested in six quadruplicate wells/plate on fifteen occasions. For each plate, the optical density (OD) value of each IQC replicate was expressed as a percentage of the median of four replicates of the C++ OD according to the ELISA data interchange software programme (EDI version 2.3.1, 1999) supplied by the FAO/IAEA. For each IQC, the preliminary upper and lower control limits of the raw absorbance signal was determined from the overall mean OD value ± 3 standard deviations (*SD*) of 90 mean OD values from 90 quadruplicates. Similarly, the tentative upper and lower control limits of the percent positivity (PP) values of each IQC was determined from the overall mean PP value ± 3 *SD* of 90 mean PP values from 90 quadruplicates.

2.5. ELISA charting methods

For the generation of Shewhart-like ELISA control data charts [11] and data processing, the spreadsheet software program Microsoft Excel for Windows '95, version 7.0, was used. Figure 1 illustrates the use of ELISA IQC data charting methods at the operator's level, which were then subjected to inter-laboratory explorative analysis. The charts were meant to visualize the agreement of the true operational performance observed under local conditions with the expected performance determined at the FAO/IAEA Agriculture and Biotechnology Laboratory to control and monitor the plate to plate, day to day and trend performance.

Internal explorative assay analysis		External explorative assay analysis
The ELISA operator generates...		The FAO/IAEA laboratory generates...
1) detailed & summary daily data charts	→	A) summary laboratory data chart and B) modified Youden plot analysis chart
2) detailed daily precision chart	→	C) summary laboratory precision chart
3) detailed daily proficiency chart	→	D) summary laboratory proficiency chart

FIG 1. Data charts generated for monitoring and evaluation of the operational ELISA performance analysing internal quality control data.

2.5.1. Detailed and summary daily data (D&SDD) chart

For each laboratory, Shewhart-like control detailed (D) & summary daily data (SDD) charts were generated to plot the daily distribution of the C++ OD values. Similar charts plotted the percentage positivity (PP) of each IQC from individual plates expressing the raw OD value as PP relative to the mean of the intermediate OD value (median OD value) of the strong positive control. Shewhart-like control charts plotted the number of plates along the x-axis against the actual absorbance values or the percent positivity values (y-axis), respectively. The upper and lower control limits (UCL-LCL) representing OD and PP mean values ± 3 *SD* were determined at the FAO/IAEA Agriculture and Biotechnology Laboratory as described under paragraph 2.4. The daily IQC results from single plates (OD and PP mean values ± 2 *SD*) and the overall mean ± 2 *SD* derived from all plates on one occasion are plotted. Some OD or PP values have been highlighted to illustrate what are extremes for the *SD*.

2.5.2. Detailed daily precision (DDPre) chart

The intra-laboratory analysis of the variation of the IQC replicates within and between plates is referred to as assay repeatability. Detailed daily precision (DDPre) charts plot the percent coefficient of variation (CV %), which is a measure of relative dispersion of IQC replicates based on the *SD*. The CV % was calculated by the *SD* of four PP replicates divided by the corresponding mean for single plates. For this study, the upper control limit was set as CV =10 %, which was empirically determined and recommended for evaluation of standardised ELISA's [15]. In addition to the CVs of C++ and C+, the DDPre chart plotted the CVs of C- and Cc. These were considered useful for monitoring but considered less meaningful for final judgement of the assay precision because their mean values approached zero.

2.5.3. Detailed daily proficiency (DDPro) chart

The detailed daily proficiency (DDPro) charts plotted the intra-laboratory assay proficiency computing the ratios of antibody binding to antibody non-binding (B/B0) of the median PPs of C+/C- from each plate. For calculation, the median of four IQC replicates rather than the mean was chosen to approach the true value rather than the value more biased by dispersion of four replicates. Also, the small difference of antibody activity of C+ compared to C- was considered more indicative to alert to reduced assay proficiency than the higher ratio of C++/C-. The tentative UCL-LCL range was determined from the overall mean value of C+/C- binding ratios ± 3 *SD* at the FAO/IAEA Agriculture and Biotechnology Laboratory (see 2.4.).

2.6. Interlaboratory explorative analysis

For each ELISA system, data of the performance indicators were generated under local conditions in laboratories in Europe and Africa. The data were reported to the FAO/IAEA Agriculture and Biotechnology Laboratory and plotted on summary data charts for explorative analysis of the ELISA performance within control limits.

2.6.1. Summary laboratory data charts

The overall IQC mean values of raw (OD) and normalised (PP) absorbance values from each laboratory representing the true data range were compared with the tentative UCL-LCL range (OD and PP mean values ± 3 SD) determined at the FAO/IAEA Agriculture and Biotechnology Laboratory.

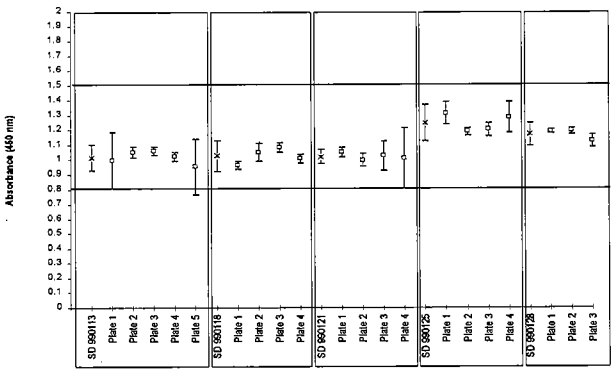

FIG. 2a. I-TAB ELISA (T.c.AGd): illustration of daily single and summary plate absorbance values plotted on a detailed and summary daily data chart at a laboratory in Africa (C++ AVG ODs ± 2 SD). Lines represent tentative upper and lower control limits (AVG ODs ± 3 SD).

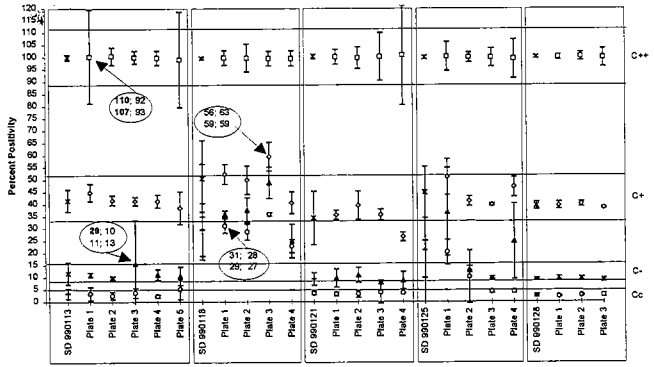

FIG. 2b. I-TAB ELISA (T.c.AGd): illustration of daily single and summary plate percent positivity (PP) values plotted on a detailed and summary daily data chart at a laboratory in Africa (C++, C+, C- and Cc AVG PPs ± 2 SD). Lines represent tentative upper and lower control limits (AVG PPs ± 3 SD). Numbers in circles represent examples of alarming PP values.

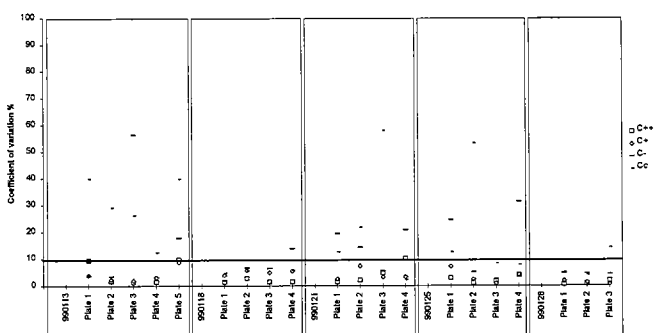

FIG. 2c. I-TAB ELISA (T.c.AGd): illustration of CV % of PP values plotted on detailed daily precision chart at a laboratory in Africa. Bold line represents tentative 10 % upper control limit.

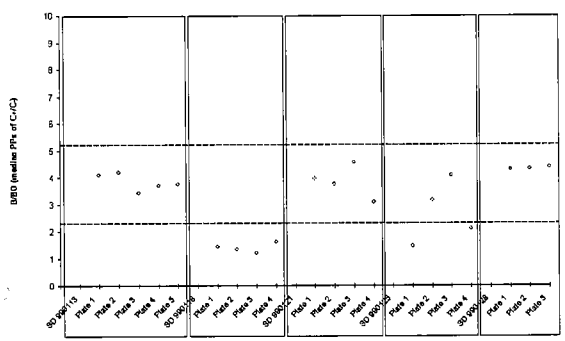

FIG. 2d. I-TAB ELISA (T.c.AGd): illustration of C+/C- binding ratios plotted from median PP values of 4 replicates each on daily detailed proficiency chart at a laboratory in Africa. --- tentative upper and lower control limit (AVG ± 3 SD).

2.6.2. Summary laboratory precision chart

For each IQC on single plates and individual laboratory, the frequency distribution of the CVs < 10 % was plotted and compared between laboratories (reproducibility). For each IQC, a box represented the true frequency range based on the overall mean of the frequency ± 1 *SD* of CVs < 10 % obtained from all laboratories.

2.6.3. Summary laboratory proficiency chart

For each laboratory, the overall mean ± 2 *SD* of binding ratios was plotted to demonstrate the intra-laboratory variation of the assay proficiency within the tentative UCL-LCL range (overall mean ± 3 *SD*) obtained from data described under paragraph 2.4. In addition, computing the overall mean ± 1 *SD* of pooled B/B0 ratios from single plates of all laboratories a range was defined to evaluate the inter-laboratory variation of the assay proficiency.

2.6.4. Modified Youden plot analysis

The modified Youden plot analysis identified systematic and random errors between laboratories [13, 14]. Briefly, a result obtained by a laboratory on one sample was plotted with respect to the result it obtained on a similar sample. Depending on the relation of the plotted point to the true value, it can be decided whether discrepant results are due to bias, imprecision, or both.

For each laboratory, the overall mean PP values of C+ (y-axis) were plotted against those of C- (x-axis). A rectangular was formed by the overall laboratory mean PP values ± 1 *SD* of C+ and C-. Laboratories reporting both IQCs outside the mean ± 1 *SD* defined quadrant indicated systematic errors (upper right or lower left region). Laboratories revealing random errors for both IQCs were visualised in the upper left or lower right region outside the mean ± 1 *SD* defined quadrant. Laboratories falling within the vertical or horizontal medium region outside the mean ± 1*SD* defined quadrant indicated a random error for 1 IQC sample.

3. RESULTS

3.1. Routine Shewhart-like charting methods of IQC data

Figures 2a to 2d illustrate Shewhart-like data charts for monitoring the ELISA under conditions in Africa. Plots of the IQC data show, at a glance, the unprocessed absorbance values of C++; the IQC PP; the CV % and B/B0 values.

Each plot shown in Figs. 2a and 2b reflects the mean value and its variation in all plates on various occasions. The bar shows the absolute range of variation of the four replicates to the mean within the measured probability on each plate and occasion. Different situations were encountered which allowed immediate interpretation of the assay performance. These were: 1) Plots showing means to be within limits and the error bars to be short and also within limits, which was ideal with reference to the IQC sample tested. 2) Plots showing the mean to be within limits, but one tail of the error bar to be out of limits. The error here was probably higher than acceptable. Reference to the individual data is recommended e.g., a single replicate could have been missed out or gave an out of limit result, which both reduced the overall mean plot and increased error. 3) Plots showing the mean, and most or all of the error bar to be outside limits. The data must be examined with reference to other IQCs, which may indicate a systematic or random error. 4) Plots showing the mean value to be below or above limits, but the error to be small, indicating little variation in results for all plates used. Similarly, occurrence of systematic or random errors needed to be carefully addressed.

Disparate situations were observed for the assay performance over time: A) All means and error bars were within limits. This indicated that the IQC values were constant and it wouldn't be expected that the test had altered in sensitivity. B) The plotted curve connecting results temporally was irregular with large "swings" in mean OD and PP values throughout time. Differences in assay variability due to frequent changes of operators might be considered. C) The plotted time curve was irregular with areas of similar means, which could have been a result of notable changes in personnel performing the assay and/or reagents. D) The curve demonstrated a fairly constant downward or upward trend irrespective of operators. This could signify altering of reagents over time.

The DDPre chart (Fig. 2c) showed estimates of the relative variation of the plotted means of IQCs from plate to plate. In the example given, the ELISA revealed CVs < 10 % for C++ and C+ indicating excellent precision. Higher CVs % were generally observed for C- and Cc as expected, even though CVs < 10 % were occasionally found.

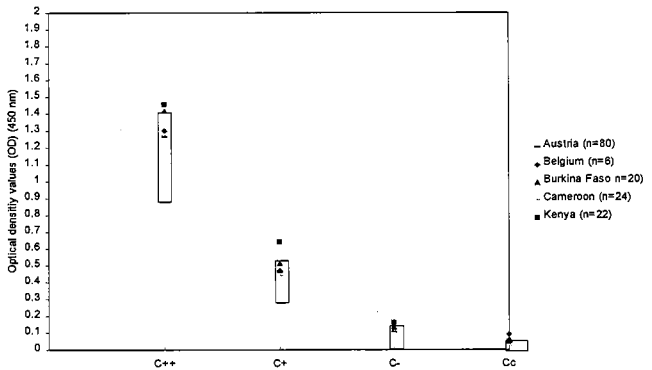

FIG. 3a. I-TAB ELISA (T.c.AGn): summary laboratory data chart plotting IQC values expressed as overall mean absorbance values. Boxes represent tentative range of upper and lower control limits (UCL-LCL) (AVG OD ± 3 SD) as determined at the FAO/IAEA Laboratory.

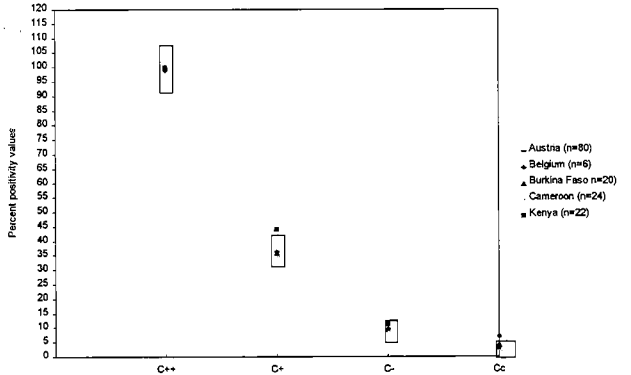

FIG. 3b. I-TAB ELISA (T.c.AGn): summary laboratory data chart plotting IQC values expressed as overall mean percent positivity values. Boxes represent tentative UCL-LCL range (AVG OD ± 3 SD) as determined at the FAO/IAEA Laboratory.

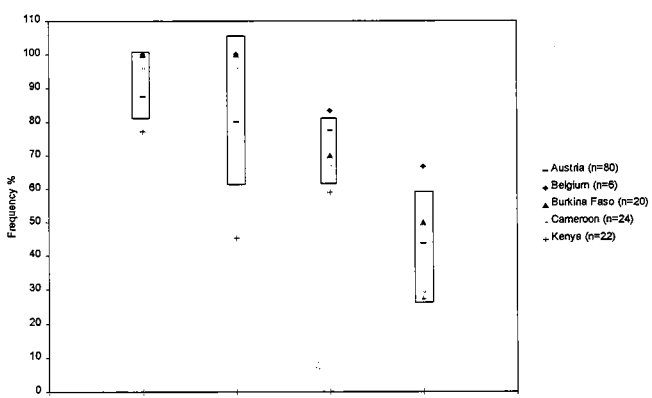

FIG. 3c. I-TAB ELISA (T.c.AGn): summary laboratory precision chart illustrating the frequency distribution of CVs < 10 %. Boxes represent the true UCL-LCL range (overall AVG ± 1 STD) obtained from all laboratories.

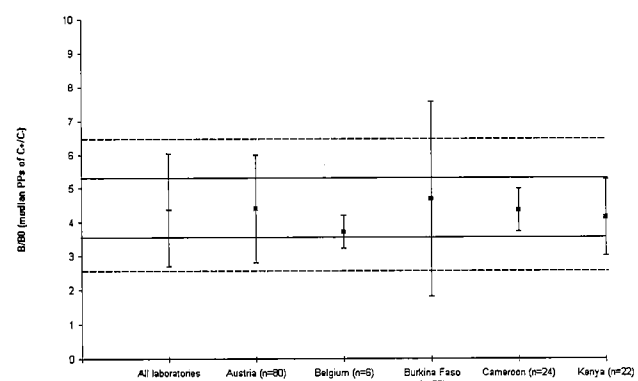

FIG. 3d. I-TAB ELISA (T.c.AGn): summary laboratory proficiency chart. - - - tentative UCL-LCL range (AVG ± 3 SD) determined at the FAO/IAEA Laboratory; — true UCL-LCL range (overall AVG ± 1 STD) obtained from all laboratories.

The DDPro chart (Fig. 2d) gave an example of the effect of systematic or random errors described above on the assay performance. The plates tested on day 990118 and two plates tested on day 990125 showed binding ratios below expected limits.

3.2. Explorative analysis of interlaboratory ELISA performance

For assessment of the ELISA performance in fifteen laboratories, the true dispersion of raw and relative absorbance, and binding ratios of IQC data were compared with tentative limits (see 2.4.). With respect to the laboratory proficiency testing, the absorbance range expressed as PP values was also explored with reference to the true data range as computed by the modified Youden plot analysis. The frequency distribution of CVs < 10 % was analysed to estimate the expected assay precision under various laboratory conditions.

3.3. I-TAB ELISA (*T.c.*AGn)

The I-TAB ELISA (*T.c.*AGn) was evaluated in three laboratories in Africa and two laboratories in Europe. In Kenya, higher absolute absorbance values for the C++, C+ and C- were observed (Fig. 3a). Similar absorbance of C++, but to a lesser extent, was observed in Burkina Faso. In Belgium and Burkina Faso, slightly more background absorbance (Cc) occurred (Fig. 3a), which did not change when PPs were plotted in the Belgium laboratory (Fig. 3b). The best assay precision was observed in Belgium and Burkina Faso, where all plates demonstrated CVs < 10 % for the C++ and C+ (Fig. 3c). A high variation of IQC replicates was observed in the Kenyan laboratory. At all laboratories, the assay proficiency was within the expected limits (Fig. 3d). The modified Youden plot analysis demonstrated that the ELISA performance under the local circumstances in Kenya was affected by systematic errors and, therefore, different to the other laboratories (Fig. 7).

In summary, for each of the four IQCs, expected absorbances were observed in two of the five laboratories. Computing PP values, three of five laboratories demonstrated controlled ELISA performance inside the established tentative limits. Comparing the assay precision between laboratories (reproducibility), four of the five laboratories demonstrated similar frequency distribution of CV < 10 % of C++ and C+ within the overall mean frequency distribution ±1 *SD*, namely 82.38 % – 101.86 % and 61.05 % - 107.46 %, respectively. The assay proficiency with respect to assay accuracy was demonstrated in all laboratories as expected. Among five laboratories, four laboratories showed similar laboratory proficiency.

3.4. I-TAB ELISA (*T.c.*AGd)

The I-TAB ELISA (*T.c.*AGd) was evaluated in twelve laboratories in Africa and two laboratories in Europe. Special attention was given to the ten laboratories where unexpected data alerted a poor ELISA performance

Ghana and Zambia consistently demonstrated higher absorbance values than expected (Fig. 4a). For Zambia, the assay performance was within expected limits on analysis of PP values (Fig. 4b). For Ghana, the high signal for C- was maintained. It was later shown that a working dilution of anti-species enzyme-conjugate of 1/14000 instead of 1/20000 was used [16]. In Mali, the C+ OD values were similar to C++ (Figs. 4a and 4b). The C- was extremely low resulting in tremendous increase of the assay proficiency (Fig. 4c). The Youden plot analysis identified that the Mali counterparts were performing the ELISA differently compared to other laboratories because of systematic errors for both the C+ and C- (Fig. 7). It was later shown that the C++ was replaced by a locally collected serum sample [17].

In Belgium and Burkina Faso a slightly higher background signal, just above the upper control limit, had to be accepted during the testing period (Figs. 4a and 4b). The latter laboratory also reported PPs of C- just above the expected limits, which was also observed in Cote d'Ivoire and Kenya.

In Uganda, the C+ was high relative to the expected C- signal (Figs. 4a and 4b) and this affected the binding ratio, which fell outside the pre-established range and approached the upper control limit based on the overall mean binding ratios ± 1 *SD* from all laboratories. The frequency of CVs < 10 % of C++ and C+ < 10 % was 50 % and 60 %, respectively, which was inferior to the frequency distribution observed in other laboratories (Fig. 4c). On Zanzibar, high overall mean absorbances outside range were observed for the weak positive and negative controls (Fig. 4a). Computing relative values, the overall mean PP of C+ was within limits, but the PP of the C- remained above the upper

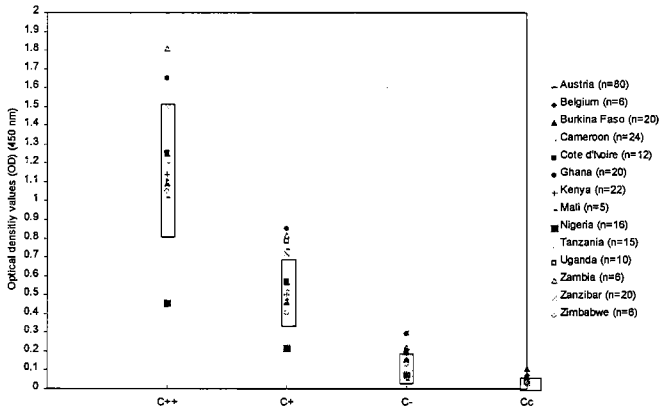

FIG. 4a. I-TAB ELISA (T.c.AGd): summary laboratory data chart plotting IQC values expressed as overall mean absorbance values. Boxes represent tentative range of upper and lower control limits (UCL-LCL) (AVG OD ± 3 SD) as determined at the FAO/IAEA Laboratory.

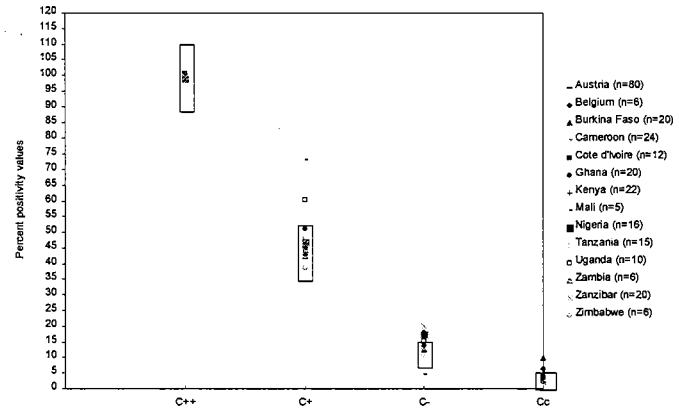

FIG. 4b. I-TAB ELISA (T.c.AGd): summary laboratory data chart plotting IQC values expressed as overall mean percent positivity values. Boxes represent tentative UCL-LCL range (AVG OD ± 3 SD) as determined at the FAO/IAEA Laboratory.

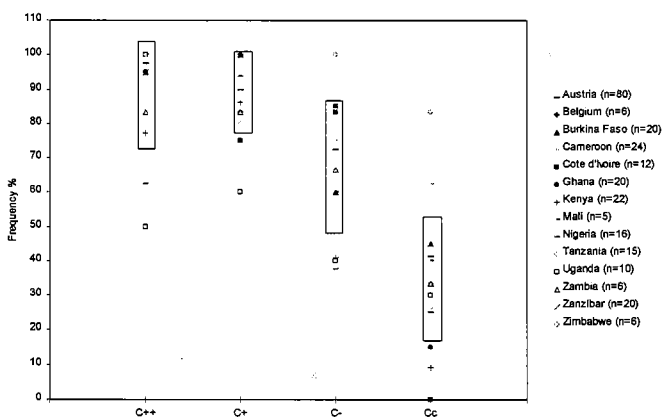

FIG. 4c. I-TAB ELISA (T.c.AGd): summary laboratory precision chart illustrating the frequency distribution of CVs < 10 %. Boxes represent the true UCL-LCL range (overall AVG ± 1 SD) obtained from all laboratories.

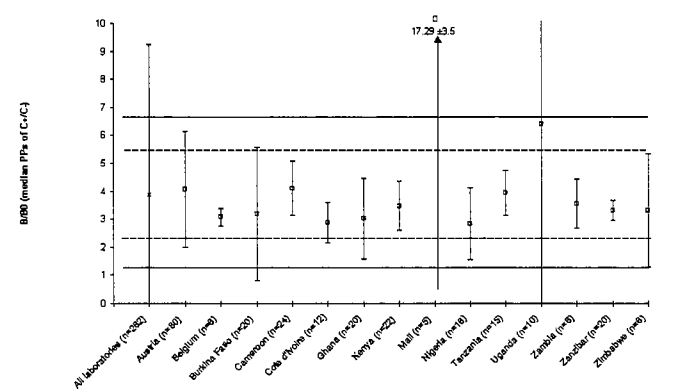

FIG. 4d. I-TAB ELISA (T.c.AGd): summary laboratory proficiency chart. - - - tentative UCL-LCL range (AVG ± 3 SD) determined at the FAO/IAEA Laboratory; — true UCL-LCL range (overall AVG ± 1 SD) obtained from all laboratories.

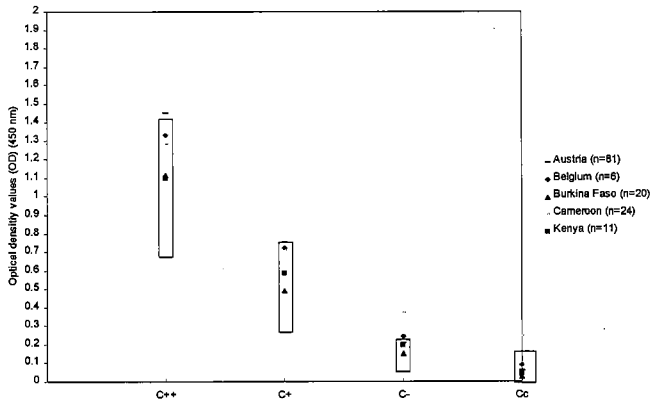

FIG. 5a. I-TAB ELISA (T.v.AGn): summary laboratory data chart plotting IQC values expressed as overall mean absorbance values. Boxes represent tentative range of upper and lower control limits (UCL-LCL) (AVG OD ± 3 SD) as determined at the FAO/IAEA Laboratory.

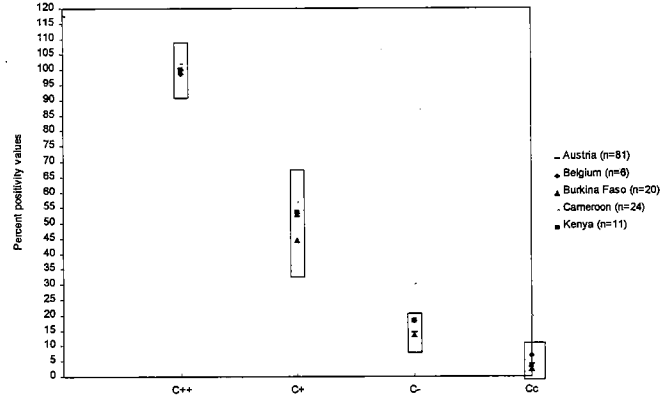

FIG. 5b. I-TAB ELISA (T.v.AGn): summary laboratory data chart plotting IQC values expressed as overall mean percent positivity values. Boxes represent tentative UCL-LCL range (AVG OD ± 3 SD) as determined at the FAO/IAEA Laboratory.

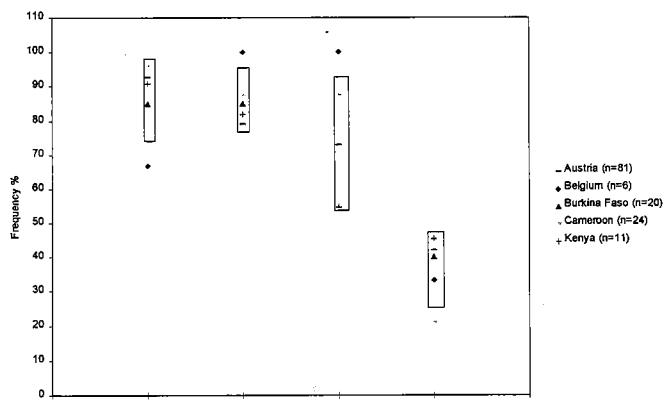

FIG. 5c. I-TAB ELISA (T.v.AGn): summary laboratory precision chart illustrating the frequency distribution of CVs < 10 %. Boxes represent the true UCL-LCL range (overall AVG ± 1 STD) obtained from all laboratories.

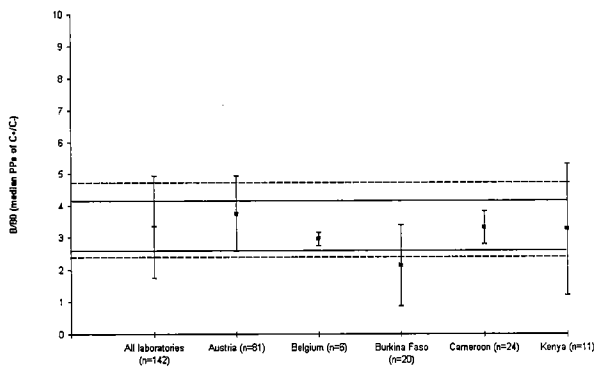

FIG. 5d. I-TAB ELISA (T.v.AGn): summary laboratory proficiency chart. - - - tentative UCL-LCL range (AVG ± 3 SD) determined at the FAO/IAEA Laboratory; — true UCL-LCL range (overall AVG ± 1 STD) obtained from all laboratories.

limit, which did not seriously affect the binding ratio (Figs. 4b and 4d). For the C+ (Uganda) and C- (Zanzibar) random errors were found (Fig. 7).

In Nigeria, the ELISA showed very low colour of C++ and C+. Expressed in PP values, the C- was unexpectedly high (Figs. 4a and 4b), without affecting the assay proficiency with respect to the assay accuracy (Fig. 4d). As observed in Uganda, the assay precision was not as good as shown in other laboratories (Fig. 4c).

In summary, for each of the four IQCs, expected absorbance and PP values was observed in five of fourteen laboratories. Similar expected assay precision was found in twelve laboratories as demonstrated by the frequency distribution of CVs < 10 % of C++ and C+ within the overall mean frequency distribution ±1 *SD* namely 73,77 % – 105.60 % and 76.87 – 100.52 %, respectively. Expected assay accuracy was obtained in twelve laboratories. Eleven of the fourteen laboratories, showed similar laboratory proficiency.

3.5. I-TAB ELISA (*T.v.*AGn)

The I-TAB ELISA (*T.v.*AGn) was evaluated in three laboratories in Africa and two laboratories in Europe. For this ELISA, the tentative limits of C++ and C+ overlapped, indicating high variation of absorbance for the antibody positive controls (C++, C+) which did not interfere with clear discrimination from C- (Fig. 5a). High absorbance of C++ was also found when the ELISA was used at the FAO/IAEA Agriculture and Biotechnology Laboratory (Fig. 5a).

Cameroon consistently gave high readings for the C- and Cc (Figs. 5a and 5b), although the C++ and C+ fell within expected limits and the diagnostic proficiency was similar to other laboratories (Fig. 5d). However, from all the five laboratories, Cameroon revealed the highest signal for C+ and C- (Fig. 7). In Belgium, the absorbance of C- was just above the upper control limit (Fig. 5a).

In Burkina Faso, the overall mean absorbance and PPs were within the expected range. However, the C+/C- binding ratio dropped just below the expected lower control limit indicating a loss of assay proficiency. This occurred due to a random error in the ELISA performance leading to reduced C+ PPs (Fig. 7). Among the five laboratories, Cameroon and Burkina Faso demonstrated an altered ELISA performance. Analysing the assay reproducibility, Belgium demonstrated 66.67 % of C++ and 100 % of C+ quadruplicates revealing CVs < 10 %.

In summary, for each of the four IQCs, expected absorbance was observed in two of the five laboratories. Absorbance expressed as PP values demonstrated controlled ELISA performance within tentative limits in four laboratories. Similar expected assay accuracy and assay precision was found in four of the five laboratories. They demonstrated a frequency distribution of CV < 10 % of C++ and C+ within the overall mean frequency distribution ±1 *SD*, namely 74,59 % – 97.81% and 78.55 % – 94.78 % respectively. Among five laboratories, three laboratories showed similar laboratory proficiency.

3.6. I-TAB ELISA (*T.v.*AGd)

The I-TAB ELISA (*T.v.*AGd) was evaluated in thirteen laboratories in Africa and two laboratories in Europe.

Nine of the total of fifteen laboratories showed unexpected assay performance. Two laboratories reported very low colour development. In Nigeria, absorbance of C++ and C+ was reduced. Data normalisation revealed PPs of C- and Cc, which were too high relative to the C+ (Figs. 6a and 6b). The binding ratio approached the lower limit of the expected range (Fig. 6d) due to a random error (Fig. 7). In Ghana, absorbances for the C++ were too low leading to unexpected high PPs of C+, C- and Cc (Figs. 6a and 6b). The binding ratio remained inside range (Fig. 6d).

Burkina Faso showed reduced overall mean PP of C+ relative to the expected overall mean absorbance of C++ dropping the assay proficiency below the lower control limit outside limits (Fig. 7). In Burkina Faso and Belgium higher backgrounds were observed. Mali and Zimbabwe reported expected overall mean absorbance of the four IQC samples. However, the overall mean PP of C+ was lower than expected indicating that the intensity of colour development varied from plate to plate.

Comparing the laboratory proficiency, among the fifteen laboratories, a discrepancy was observed in Mali and Burkina Faso due to random errors for C+; in Nigeria, a random error encountered for C-, and in Ghana a systematic error affected C+ and C- (Fig. 7).

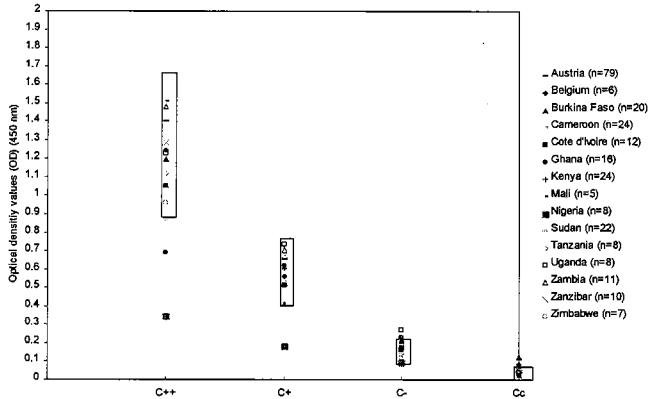

FIG. 6a. I-TAB ELISA (T.v.AGd): summary laboratory data chart plotting IQC values expressed as overall mean absorbance values. Boxes represent tentative range of upper and lower control limits (UCL-LCL) (AVG OD ± 3 SD) as determined at the FAO/IAEA Laboratory.

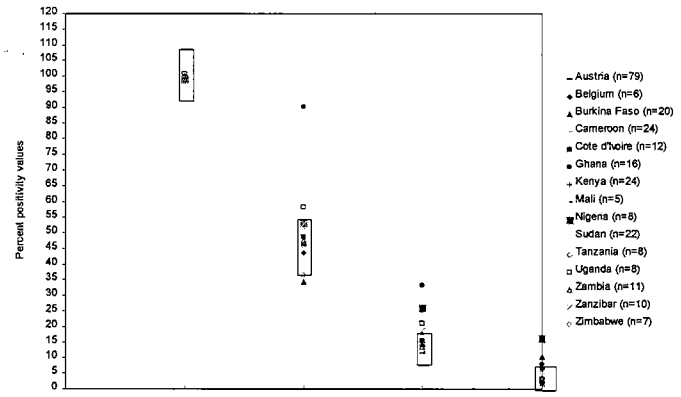

FIG. 6b. I-TAB ELISA (T.v.AGd): summary laboratory data chart plotting IQC values expressed as overall mean percent positivity values. Boxes represent tentative UCL-LCL range (AVG OD ± 3 SD) as determined at the FAO/IAEA Laboratory.

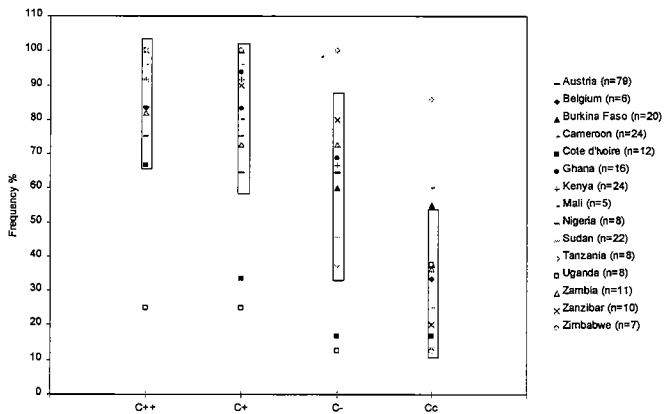

FIG. 6c. I-TAB ELISA (T.v.AGd): summary laboratory precision chart illustrating the frequency distribution of CVs < 10 %. Boxes represent the true UCL-LCL range (overall AVG ± 1 STD) obtained from all laboratories.

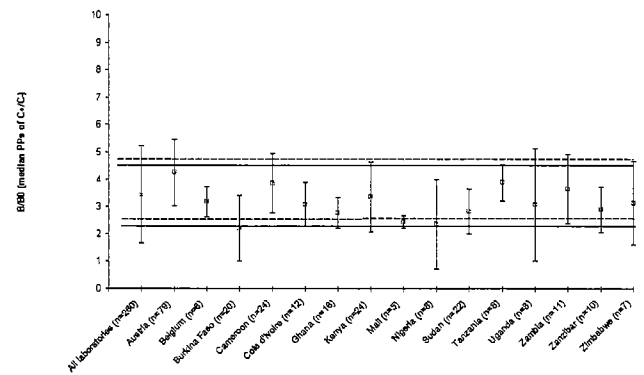

FIG. 6d. I-TAB ELISA (T.v.AGd): summary laboratory proficiency chart. - - - tentative UCL-LCL range (AVG ± 3 SD) determined at the FAO/IAEA Laboratory; — true UCL-LCL range (overall AVG ± 1 STD) obtained from all laboratories.

In Uganda unexpected high overall mean PPs of C+ and C- were found without effecting the binding ratios. Estimating the assay repeatability, CVs < 10 % of C++ and C+ quadruplicates were only rarely found (25 %). The assay performance on Zanzibar was characterised by an overall mean absorbance and PP value of C- above the tentative upper limit, which did not affect the assay proficiency. In Sudan, the assay revealed marginally less absorbance, which did not affect the assay proficiency.

In summary, for each the four IQCs, expected absorbance was observed in eight of fifteen laboratories and expected PP values were observed in seven of fifteen laboratories. Analysing the interlaboratory assay precision, fourteen and thirteen laboratories demonstrated similar frequency distributions of CV < 10 % of C++ and C+, respectively, within the overall mean frequency distribution ±1 *SD*, namely 66.30 % – 106.21 % and 56.74 % - 103.96 %, respectively. Expected assay proficiency was observed in twelve laboratories. Among fifteen laboratories, eleven laboratories showed similar laboratory proficiency.

4. DISCUSSION

The use of charting methods to plot standardised IQC data on Shewhart-like data control charts proved useful to monitor and evaluate the operational performance of indirect ELISA methods forthe detection of trypanosomal antibodies. The charting methods: i) kept a constant record of all data, ii) monitored the ELISA's from day to day, and week to week, iii) rapidly identified unacceptable results, iv) helped to determine problems with specific reagents, v) noted trends in results, e.g., a decrease in performance, vii) identified bias of ELISA performance due to different operators, and vii) fulfilled various criteria for good laboratory practice (GLP). In addition, the establishment of standardised and transparent IQC data charting methods for quality control of the ELISA performance should provide a measure of confidence to national laboratory proficiency with respect to reports on disease occurrence.

The evaluation of the operational performance of four indirect ELISA systems in fifteen laboratories demonstrated that the majority reported similar laboratory proficiency for the I-TAB ELISA (*T.c.*AGn) (four of five laboratories), I-TAB ELISA (*T.c.*AGd) (eleven of fourteen laboratories), I-TAB ELISA (*T.v.*AGn) (three of five laboratories), I-TAB ELISA (*T.v.*AGd) (eleven of fifteen laboratories. These findings were in agreement with those obtained from a previous pilot study [18]. The data reported here and those with reference to the diagnostic sensitivity and robustness of ELISA method [9, 10, 19] provided evidence that the ELISA's exploiting heat-denatured antigens of *T. congolense* and *T. vivax* can be successfully used for trypanosomosis control following transfer to diagnostic laboratories in the tropics.

The detailed plate to plate analysis of the replicates of C++, C+, C- and Cc allowed comparison with the defined population data determined at the FAO/IAEA Agriculture and Biotechnology Laboratory, so that unexpected absolute (OD) and relative absorbance (PP) values alerted operators to problems in good time. The discrepancies between the test absorbance of IQCs result and specified absorbance was attributed to unavoidable random errors inherent in every measurement procedure. The factors that influence the outcome of a measurement can not all be completely controlled. In the practical interpretation of ELISA measurement data, this variability has to be taken into account and requires additional explorative analysis. Therefore, the method of using binding ratios for C+/C- was analysed to allow comparison of the analytical sensitivity with respect to the accuracy of assessing that a sample was positive both within and between tests in laboratories. The frequency distribution of CVs < 10 % of C++ and C+ PP values, indicative of the assay precision, was also examined. It was demonstrated that the assays performed reasonably well within the true range represented by overall mean value ± 1 *SD* of all laboratories. This suggested that the ELISA systems were similarly affected by uncertainties occurring at individual laboratories which can not be controlled by the production site of the ELISA's.

It should be noted that the results of IQCs performance indicators such as reduced binding ratios do not automatically control the assay proficiency with reference to the diagnostic sensitivity and specificity. Here, re-testing of test serum samples would be recommended or even better the consistent plate to plate analysis of defined positive and negative reference sera representing the studied population.

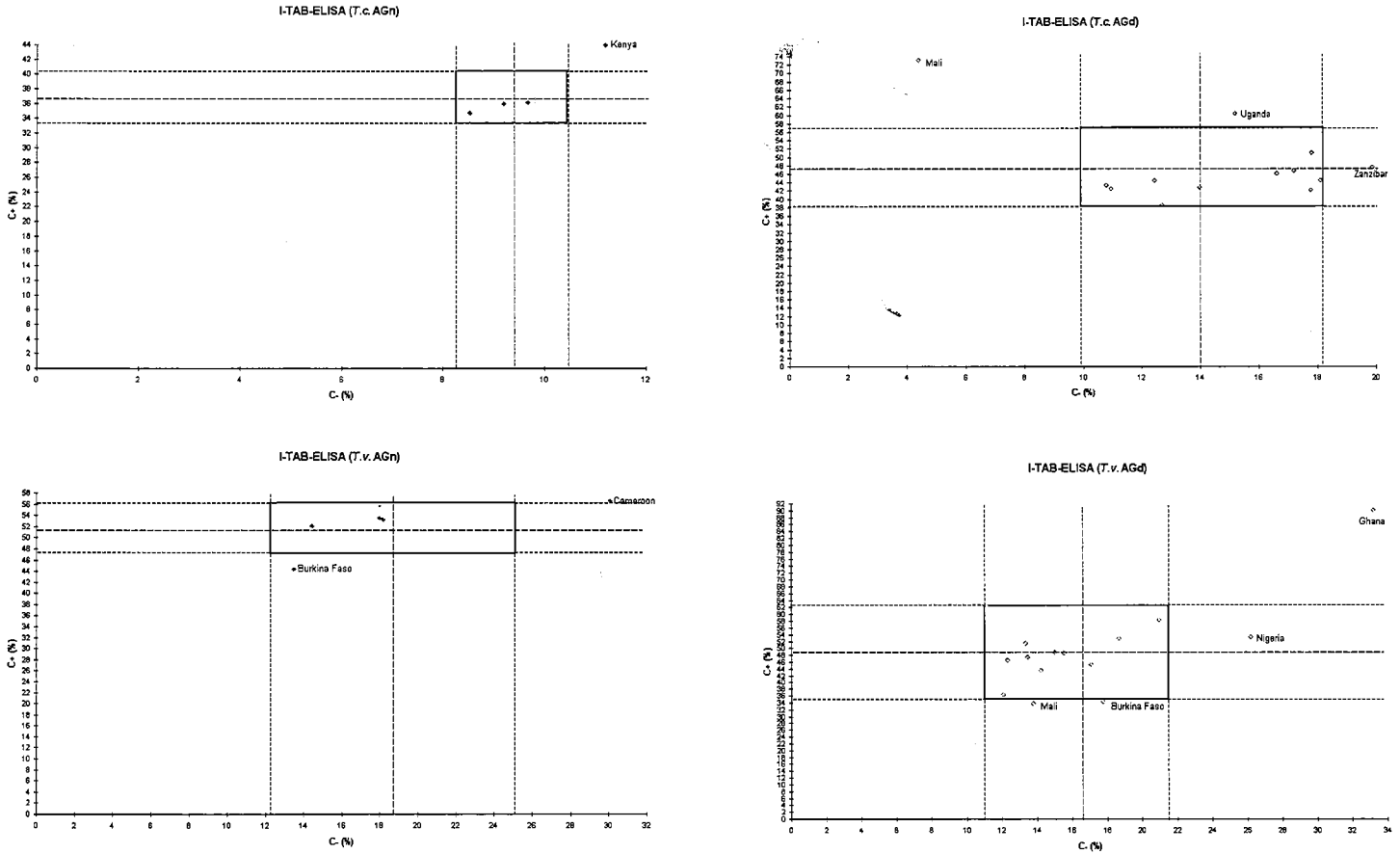

FIG. 7. Results of modified Youden plot analysis used for laboratory proficiency testing. Using indirect antibody detection ELISAs for trypanosomosis random and systematic errors in laboratories were identified. For I-TAB ELISA (T.c.AGn), I-TAB ELISA (T.c.AGd) and TAB I-ELISA (T.v.AGd) note that data within range overlapped with results from one additional laboratory. For further explanation see under MATERIALS AND METHOD.

From the data it became evident that the sole use of the IQC absorbance range did not truly reflect the potential ELISA performance and should, therefore, not be used as the only decision criteria for plate acceptance or rejection. It is proposed to refer also to the assay precision and binding ratios, which can be easily recorded on Shewhart-like data charts at individual laboratories. For interlaboratory evaluation of the ELISA performance, data should then be reported to the ELISA production site, responsible for continuous control and monitoring of the performance of ELISA systems.

In conclusion, a control quality procedure was established for evaluation of the operational performance of indirect trypanosomosis ELISA method within and between laboratories. The results provided a measure of confidence in the reliable use of trypanosomosis antibody ELISA's with reference to controlled assay performance in diagnostic laboratories in Africa and Europe. The trypanosomosis ELISA method was therefore considered "fit for purpose".

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