

Original Article

## Field accuracy of HIV rapid diagnostic tests for blood donor screening, Bukavu, Eastern Democratic Republic of the Congo

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### Abstract

**Introduction:** Rapid diagnostic tests (RDTs) are widely used for point-of-care diagnosis of HIV infection in resource-limited settings. However, there are no data about their field diagnostic performance in Eastern Democratic Republic of the Congo (DRC), especially in the context of blood banks screening for transfusion safety purpose.

**Methodology:** Blood specimens were collected from blood donors in Bukavu, Eastern DRC, from May the 1<sup>st</sup> to June the 30<sup>th</sup>, 2015, to evaluate the accuracy of Alere Determine HIV-1/2, Trinity Biotech Uni-Gold HIV, and DoubleCheckGold Ultra HIV 1&2 compared to the laboratory-based 4<sup>th</sup> generation ELISA apDia HIV Ag/Ab assay. Sensitivity, specificity, positive and negative predictive values, and related 95% confidence intervals were calculated using MedCalc statistical software version 15.1. Reliability was evaluated using Cohen's Kappa Statistic,  $\kappa$ .

**Results:** Of 312 participants who donated blood, 96/312 (30.7%) were female and the mean age (SD) was 31.7 years ( $\pm$  8.1 years). Sensitivity for the three tests was 57.1% (95% CI: 18.4-90.1). The specificity was 99.7% (95% CI: 18.4-90.1) for Alere Determine HIV-1/2, 100% (95% CI: 98.8-100.0) for Uni-Gold HIV, and 100% (95% CI: 98.8-100.0) for DoubleCheckGold Ultra HIV 1&2. Cohen's Kappa Statistic showed moderate agreement between the 4<sup>th</sup> generation ELISA apDia HIV Ag/Ab and either Alere Determine HIV-1/2 and Uni-Gold HIV ( $\kappa$  = 0.66; 95% CI: 0.55- 0.76) but good agreement for DoubleCheckGold Ultra HIV1&2 ( $\kappa$  = 0.72; 95% CI: 0.61 – 0.82).

**Conclusions:** Compared to the laboratory-based ELISA apDia HIV Ag/Ab assay, the currently used 3<sup>rd</sup> generation HIV RDTs showed poor field accuracy results for blood donor screening. These data support the need for 4<sup>th</sup> generation Ag-Ab RDTs in transfusion blood qualification.

**Key words:** HIV; Rapid Diagnostic Tests; Accuracy; DR Congo.

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### Introduction

The detection of antibodies against human Immunodeficiency Virus (HIV) through commercially available 3<sup>rd</sup> generation rapid diagnostic tests (RDTs) has been performed globally for more than a decade with high acceptability, feasibility and an affordable cost [1,2]. Indeed, these RDTs can be stored at ambient temperature and are typically easy to use at point-of-care (POC) in a variety of clinical and public health settings such as HIV diagnosis in adults, screening of pregnant women and infants, and blood collected from healthy volunteer donors as well as for surveillance

purposes. Furthermore, high sensitivity and specificity have been reported by their manufacturers for licensing purposes and subsequent World Health Organization (WHO) prequalification [3-5]. However, these HIV RDTs have some limitations since they cannot identify persons who are within the window period of an acute HIV infection due to the fact that these individuals have not yet developed HIV-specific antibodies [6,7]. Such individuals are highly infectious due to concurrent high plasma as well as vaginal and semen HIV-1 viral load [8-9]. Identification of acute HIV infection requires detection of HIV nucleic acids or p24 antigens. New 4<sup>th</sup>-

generation laboratory-based assays for HIV-1 detect HIV-1 p24 antigenaemia as well as antibodies to HIV-1/2 [10-14].

In countries with a relatively low HIV prevalence ( $\leq 5\%$ ) such as the Democratic Republic of the Congo (DRC), WHO recommends that blood samples are first tested with one 3<sup>rd</sup> generation RDT assay (e.g. Alere Determine™ HIV-1/2), and specimens that are non-reactive are reported as negative. Re-testing of such non-reactive individuals within 4 to 6 months is usually recommended, especially for at-risk individuals (e.g. key populations). Specimens that are reactive on the first-line assay require confirmatory testing using additional, different assays [15].

Failure to diagnose HIV in the weeks after an individual has acquired infection raises important safety issues in the context of blood transfusion and also negatively impacts the epidemic control by increasing the likelihood of onward transmission [9]. Furthermore, there are little data about real-life accuracy of HIV RDTs in DRC in the context of blood transfusion safety, and other factors not related to the performance of HIV RDTs in these settings may impact their accuracy. Therefore, we aimed to investigate the diagnostic performance of several 3<sup>rd</sup> generation RDTs versus a 4<sup>th</sup> generation enzyme-linked immunosorbent assay (ELISA) apDia HIV Ag/Ab from blood samples collected from volunteer donors.

## Methodology

### *HIV RDTs Evaluated, Comparator and Sample Collection*

We evaluated the performance of the following RDT assays: Alere Determine HIV-1/2 (Alere Medical Co. Ltd., Chiba-ken Japan), Uni-Gold HIV (Trinity Biotech, plc, Bray Co, Wicklow, Ireland) and DoubleCheckGold Ultra HIV 1&2 (Orgenics Ltd, Yavne, Israel). We used the 4<sup>th</sup> generation ELISA apDia HIV Ag/Ab as assay for comparison.

Three hundred and twelve blood transfusion bags were collected from the same number of blood donors from May 1<sup>st</sup> to June 30<sup>th</sup>, 2015 at five urban or rural community health training centers or “formations sanitaires” (FOSA) in Bukavu City, DRC. FOSA sites included: Provincial General Referral Hospital of Bukavu, Panzi General Referral Hospital, General Referral Hospital of Chiriri, General Referral Hospital of Nyantende, and the Provincial Health Center for Blood Transfusion. We centrifuged 10 ml of bag blood samples at 3000 rpm for 5 minutes. Study samples were transported in an isothermal box at 4 °C and stored at -20 °C at the Medical School laboratory of Université

Evangelique en Afrique (UEA) for processing. We used SeraCare Sciences Control Panels (Panel AZ HIV-1 Seroconversion) [16] which included AZ PRB 947, AZ PRB 950 and AZ PRB 955. A total of 21 sera from these panel were tested. The results of these panels were compared to results obtained by the Institute of Tropical Medicine (ITM) of Antwerp, Belgium, using similar panels during its 2011 evaluation of the apDia HIV Ag/Ab ELISA (ITM evaluation report: apDia HIV Ag/Ab ELISA Apr 2011, unpublished).

### *Laboratory Assays*

Testing procedures were conducted, according to manufacturers' instructions and independently, by two different qualified laboratory technicians. In the event of a conflicting result, a further test was carried out by a third laboratory technician for a final decision. Then, the samples were tested using the 4<sup>th</sup> generation ELISA apDia HIV Ag/Ab. We used the «cutoff» method and the Elisa Micro Read Code 1000 and MICRO Wash Code 1100 equipment from Global Diagnostics (Gel, Belgium). All initially reactive samples were re-tested with the same assay once as recommended by the manufacturer. No discrepancies were observed between the 1<sup>st</sup> and the 2<sup>nd</sup> test result for initially reactive samples.

### *Sample Size and Power Calculations*

Based on Bujang and Adnan, we estimated that a total sample size (N) of 312 blood samples testing under pre-specified values of power (80%), type I error (5%) and assuming an effect size determined by the HIV prevalence in our study setting (5%), and anticipated values of both sensitivity (55%), specificity (85%) of the RDTs under evaluation for both null ( $H_0$ ) and alternative ( $H_a$ ) hypotheses, respectively (Prevalence=0.05,  $H_0 = 0.55$  and  $H_a = 0.85$ ), to obtain a minimum number (N1) of true HIV+ tests (N1 = 15) [17].

### *Statistical Analysis*

Results are reported in accordance with the Standards for Reporting of Diagnostic Accuracy checklist [18]. Sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV and NPV), and 95% confidence intervals (CI), as well as Cohen's Kappa statistic ( $\kappa$ ), were calculated for each antibody assay using the MedCalc version 15.1 (MedCalc Software, Ostend, Belgium) [19]. Kappa test ( $\kappa$ ) measured inter-rater agreement between the three RDTs under evaluation and the 4<sup>th</sup> generation apDia HIV Ag/Ab assay, in which a Kappa value of 0 indicated less

**Table 1.** RDTs accuracy and reliability results.

ApDia HIV Ag/Ab 4 <sup>th</sup> gen. (Reference Test)	n	Alere Determine HIV-1/2		Trinity Biotech Uni-Gold		Double check Gold Ultra HIV 1&2	
		Positive	Negative	Positive	Negative	Positive	Negative
Results							
Positive	7	4	3	4	3	4	3
Negative	305	1	304	1	304	0	305
Se % (95% CI)		57.1 (18.4-90.1)		57.1 (18.4-90.1)		57.1 (18.4-90.1)	
Sp % (95% CI)		99.6 (98.1-99.9)		99.6 (98.1-99.9)		100 (98.8-100)	
PPV % (95% CI)		80.0 (28.3-99.4)		80.0 (28.3-99.4)		100 (39.8-100)	
NPV % (95% CI)		99.0 (97.1-99.8)		99.0 (97.1-99.8)		99.0 (97.1-99.8)	
PLR (95% CI)		174.3 (22.2-1366.4)		174.3 (22.2-1366.4)		-	
NLR (95% CI)		0.43 (0.20-1.01)		0.43 (0.20-1.01)		0.43 (0.18-1.01)	
Kappa (weighted)		0.66 (0.34-0.97)		0.66 (0.34-0.97)		0.72 (0.42-1.00)	

Se: Sensitivity; Sp: Specificity; PPV: Positive predictive value; NPV: negative predictive value; PLR: Positive Likelihood Ratio; NLR: Negative Likelihood Ratio.

than chance agreement, and a value of 0.99 indicated perfect agreement.

### Regulatory Approvals

The study was approved by the Ethics Research Committee of the Catholic University of Bukavu, DRC (Approval Reference No. UCB/CIE/NC/012/2015).

## Results

### Overall Descriptive Data

Of 312 participants who donated blood, 96/312 (30.7%) were female and the mean age (SD) was 31.7 years ( $\pm$  8.1 years). Seven blood donors (2.2%) were found to be HIV-positive by the 4<sup>th</sup> generation ELISA apDia HIV Ag/Ab assay. In contrast, only 5/312 (1.6%), 5/312 (1.6%), and 4/312 (1.3%) samples tested positive by Alere Determine HIV-1/2, Uni-Gold HIV, and DoubleCheckGold Ultra HIV 1&2, respectively (Table 1). When compared to the 4<sup>th</sup> generation ELISA apDia HIV Ag/Ab assay, there were two HIV+ samples that were missed (“false negative”) by all three RDTs under evaluation, but only one sample had a reactive result (“false positive”) by Alere Determine HIV-1/2 and Uni-Gold HIV which was not confirmed by the 4<sup>th</sup> generation ELISA apDia HIV Ag/Ab.

### Test Accuracy

As shown in Table 1, Alere Determine HIV-1/2, Uni-Gold Ultra HIV, and DoubleCheckGold Ultra HIV 1&2 had the same Sensitivity: 57.1% (95% CI: 18.4-

90.1), while the specificities for Alere Determine HIV-1/2 and Uni-Gold HIV were the same (99.7%; 95% CI: 18.4-90.1) and similar (100.0%; 95% CI: 98.8-100.0) to DoubleCheckGold Ultra HIV 1&2. Negative predictive values (%; 95% CIs) were 99.0 (97.1-99.8); 99.0 (97.1-99.8); and 99.0 (97.1-99.8) for Alere Determine HIV-1/2, Uni-Gold Ultra HIV, and DoubleCheckGold Ultra HIV 1&2, respectively. The results of the Control Test Panels are shown in Table 2, with the 4<sup>th</sup> generation ELISA apDia HIV Ag/Ab assay demonstrating high sensitivity and maximum positive predictive values.

### Inter-measurement agreement (Cohen’s Kappa Statistic, $\kappa$ )

Kappa statistic showed moderate agreement between the 4<sup>th</sup> generation ELISA apDia HIV Ag/Ab and RDTs Alere Determine HIV-1/2 and Uni-Gold HIV ( $\kappa$  = 0.66; 95% CI: 0.55- 0.76) and good agreement for apDia and DoubleCheckGold Ultra HIV 1&2 ( $\kappa$  = 0.72; 95% CI: 0.61 – 0.82) (Table 1).

## Discussion

We found an unacceptably low sensitivity yet high specificity, and a moderate agreement for all three HIV RDTs evaluated, when compared to the 4<sup>th</sup> generation ELISA apDia HIV Ag/Ab assay from blood samples collected from blood donors for transfusion purposes. These results are in line with findings from Ethiopia by

**Table 2.** Control panel results.

Control Panel	n	Alere Determine HIV-1/2		Trinity Biotech Uni-Gold		Double check Gold Ultra HIV 1&2			ApDia HIV Ag/Ab 4 <sup>th</sup> gen.	
		Positive	Negative	Positive	Negative	Positive	Indeterminate	Negative	Positive	Negative
Results										
Positive	10	5	5	5	5	2	3	5	10	0
Negative	3	0	3	0	3	0	0	3	0	3
Se (95% CI)		50.0 (18.7-81.3)		50.0 (18.7-81.3)		50.0 (18.7-81.3)			100 (69.2-100)	
NPV (95% CI)		37.5 (8.5-75.5)		37.5 (8.5-75.5)		37.5 (8.5-75.5)			100 (29.2-100)	

Se: Sensitivity; NPV: Negative predictive value.

Dessie *et al.* in which Alere Determine HIV-1/2 showed low sensitivity (60.5%) [20].

Our study highlights the important issue of possible false-negative HIV test results, which in part may be explained by the failure of these RDTs to diagnose HIV during the window period. During this period, HIV infection can be detected only by tests detecting also viral antigen such as the laboratory-based 4<sup>th</sup> generation ELISA apDia [6]. Also, it is worth noting and somewhat assuring that in a context of this low-risk study population of blood donors and in an already low HIV prevalence setting such as the South Kivu province of DRC, the NPV HIV RDTs is very high (99%), as documented in our results. Therefore, the baseline HIV prevalence in the population influences the negative and PPV and needs to be taken into account in the interpretation of HIV RDTs results. On the other hand, it is not certain that the currently available 4<sup>th</sup> generation HIV RDTs would perform better based on recent published data showing that the HIV p24 antigen detection component of some 4<sup>th</sup> generation RDTs also lacks analytical and diagnostic sensitivity [21-25].

It seems unlikely that the false-negative samples missed by HIV RDTs in our study, but which were positive for p24 antigen and/or HIV antibodies by the 4<sup>th</sup> generation ELISA apDia HIV Ag/Ab assay, might represent very early infections (p24 antigen only) or somewhat later ones (with antibodies at a low level, not detectable by the RDTs). Given the qualitative results generated by the laboratory-based 4<sup>th</sup> generation ELISA apDia test used and the lack of resources for p24 antigen or nucleic acid testing (NAT) in Eastern DRC, we could not resolve this uncertainty.

Other possible causes of false-negative tests include divergent HIV sub-types, a possible scenario in DRC which is known for a high prevalence of recombinant HIV strains [26]. It is also worth mentioning the possibility of human error, such as the addition of insufficient specimen or too much buffer when procedures are handled by non-trained or unsupervised staff [27], transportation or storage of test kits outside of recommended conditions, leading to possible denaturation of reagents or test devices, and the use of expired reagents or test devices [26]. Of note, these latter factors were unknown in our study but would add to potential problems in real-life situations in developing countries.

The results of this study have several important clinical and public health implications. First, these data raise serious concerns about blood transfusion safety and call for stringent quality control measures and the need for laboratory-based antigen testing or NAT if

acute HIV infection is suspected. Second, in the era of test-and-treat strategies, there is a need to use and scale-up highly sensitive and specific HIV RDTs to optimize the first and second steps in the HIV care cascade (diagnosis and linkage to care) if the 90-90-90 UNAIDS 2020 targets are to be reached. The use and scale-up of emerging low cost and point-of-care tests, such as the highly sensitive and easy-to-handle molecular HIV diagnostic tests Xpert HIV-1 Qualitative and Xpert HIV-1 Viral Load Test (e.g. Alere qHIV1/2 detect), would represent a significant step forward in our efforts towards early HIV diagnosis. These automated HIV diagnostic platforms do not require much infrastructure and can be used at or near point-of-care. However, they are more expensive and efforts need to be made in the future through public-private partnership to make them affordable and accessible in sub-Saharan Africa where they are badly needed [28-30].

While informative, our study also has some limitations. Indeed, given the low-risk study population and the low HIV prevalence setting, we were not able to obtain sufficient true HIV positive tests [18] for robust conclusions. Therefore, to overcome these limitations, we recommend that future studies make an effort to recruit sufficient true positive HIV tests, which is likely to be achieved by using a higher HIV prevalence study population. This will lead to more robust conclusions about field accuracy of these HIV RDTs in the specified context on interest. Finally, despite the above limitations, we believe that our study reports and discusses results, challenges, and future paths about the accuracy and reliability of HIV RDTs. This is of clinical and public health importance and makes a reasonable contribution to the field.

## Conclusions

Our study raised the possibility of unacceptably low sensitivities of the RDTs Alere Determine HIV-1/2, Uni-Gold HIV, and DoubleCheckGold Ultra HIV 1/2 using blood samples from blood donors in Eastern DRC. This raises serious concerns about blood transfusion safety and calls for stringent quality control measures as well as the need for 4<sup>th</sup> generation Ag-Ab RDTs and a cross-evaluation of blood transfusion samples by a laboratory-based antigen testing, whenever feasible. These data need to be confirmed by further research with well-powered studies and in a high HIV prevalence resource-limited setting.

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### Authors Contributions

MKT conceived the study and drafted the first draft of the manuscript. MKT, KB and MPM conducted the statistical analyses. All authors contributed on the data interpretation, writing, and approval of the final version of the manuscript.

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