# COMPARING THE EFFECTIVENESS OF DIFFERENT HIV TESTING TECHNIQUES USED IN ZAMBIA; A SYSTEMATIC REVIEW OF DATA FROM A CLINICAL PERFORMANCE STUDY.


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## ABSTRACT


**BACKGROUND**

According to UNAIDS, HIV/AIDS, is one of the world's most serious public health challenges. Even though there has been tremendous global commitment and improvement to stopping new HIV infections and ensuring that everyone with HIV has access to treatment in the last 3 decades, the journey to curb the HIV epidemic has been very slow. Despite the availability of a widening array of effective HIV prevention tools and methods and a massive scale-up of HIV treatment in recent years, UNAIDS cautions that there has been unequal progress in reducing new HIV infections (UNAIDS, 2019). The purpose of this study is to investigate the effectiveness of different HIV testing strategies used in Zambia, to show the accuracy of each test in order to examine if the addition of a 4th generation RDT to the algorithm would help reduce HIV incidence.

**METHODS**

The study used a quantitative descriptive approach to analyze samples of 2564 participants, of ages 15 to 95 years from two Lusaka sites. Using a systematic analysis technique, quantitative methods were applied to evaluate different variables and compare them against each other to find relationships. The data was analyzed using Microsoft excel and statistical software SPSS version 25.0.

**RESULTS**

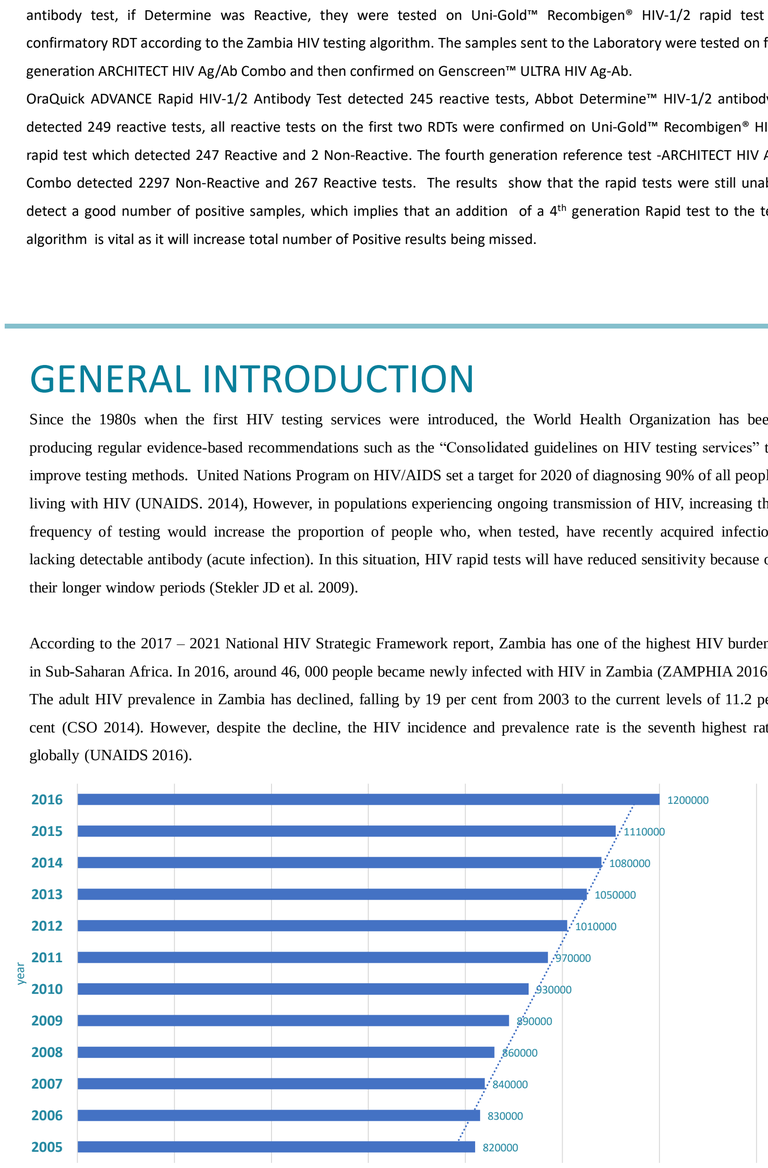
2564 participants were tested on OraQuick ADVANCE Rapid HIV-1/2 Antibody Test and Abbot Determine™ HIV-1/2 antibody test, if Determine was Reactive, they were tested on Uni-Gold™ Recombigen® HIV-1/2 rapid test as a confirmatory RDT according to the Zambia HIV testing algorithm. The samples sent to the Laboratory were tested on fourth generation ARCHITECT HIV Ag/Ab Combo and then confirmed on Genscreen™ ULTRA HIV Ag-Ab.

OraQuick ADVANCE Rapid HIV-1/2 Antibody Test detected 245 reactive tests, Abbot Determine™ HIV-1/2 antibody test detected 249 reactive tests, all reactive tests on the first two RDTs were confirmed on Uni-Gold™ Recombigen® HIV-1/2 rapid test which detected 247 Reactive and 2 Non-Reactive. The fourth generation reference test -ARCHITECT HIV Ag/Ab Combo detected 2297 Non-Reactive and 267 Reactive tests. The results show that the rapid tests were still unable to detect a good number of positive samples, which implies that an addition of a 4th generation Rapid test to the testing algorithm is vital as it will increase total number of Positive results being missed.


## GENERAL INTRODUCTION

Since the 1980s when the first HIV testing services were introduced, the World Health Organization has been producing regular evidence-based recommendations such as the "Consolidated guidelines on HIV testing services" to improve testing methods. United Nations Program on HIV/AIDS set a target for 2020 of diagnosing 90% of all people living with HIV (UNAIDS. 2014), However, in populations experiencing ongoing transmission of HIV, increasing the frequency of testing would increase the proportion of people who, when tested, have recently acquired infection lacking detectable antibody (acute infection). In this situation, HIV rapid tests will have reduced sensitivity because of their longer window periods (Stekler JD et al. 2009).

According to the 2017 – 2021 National HIV Strategic Framework report, Zambia has one of the highest HIV burdens in Sub-Saharan Africa. In 2016, around 46, 000 people became newly infected with HIV in Zambia (ZAMPHIA 2016). The adult HIV prevalence in Zambia has declined, falling by 19 per cent from 2003 to the current levels of 11.2 per cent (CSO 2014). However, despite the decline, the HIV incidence and prevalence rate is the seventh highest rate globally (UNAIDS 2016).




Source: CSO 2016.
People living with HIV in Zambia per year

Approximately there are 1.2 million people (11.1%) in Zambia living with HIV (UNAIDS 2016). Zambia has a generalized HIV epidemic among adults aged 15-49 years with more women (13.6%) than men (8.5%) living with the virus (CSO 2014). Data from the ZDHS indicates that the Zambian HIV epidemic is geographically heterogeneous with provincial prevalence rates ranging from 4.8 per cent to 15.1 per cent. HIV prevalence rates among men and women aged 15-49 years are higher in urban (15.6 per cent) compared to rural (7.4 per cent) areas (CSO 2014).

## PROBLEM STATEMENT

Identification of acute HIV infection requires detection of HIV nucleic acids or p24 antigens which is possible with 4th generation laboratory-based assays that detect HIV-1 p24 antigen as well as antibodies to HIV-1/2 (Mitchell EO et al 2013; Bentsen C et al 2011; Nasrullah M et al 2013; Chavez P et al 2011; Fiebig EW et al 2003).

In low HIV prevalence countries (≤ 5%), WHO recommends that blood samples are first tested with one 3rd generation RDT assay (e.g., Alere Determine™ HIV-1/2), and specimens that are non-reactive are reported as negative. These non-reactive individuals are recommended to retest within 4 to 6 months which rarely happens. Specimens that are reactive on the first-line assay require confirmatory testing using additional and different assays (WHO 2015).

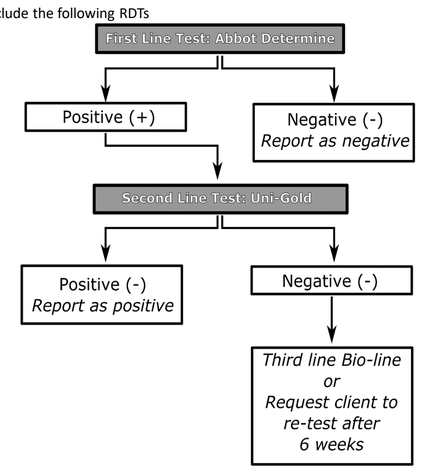
Since the beginning of the HIV/AIDS epidemic there have been a number of studies conducted in different countries and under different clinical settings that have shown that Rapid Diagnostic tests (RDTs) are specific and sensitive enough to be be used for screening and diagnosing HIV. When these RDTs are compared to laboratory based fourth generation tests, they fail to detect acute infections (Patel P et al 2010).

Most studies on HIV RDT kits report varying sensitivity and specificity with test specimen types. The evidence from these studies suggests a need to assess HIV RDT kits used in specific settings to determine the blood-based specimen type that improves performance (Boadu et al., 2016).

## ZAMBIA HIV TESTING ALGORITHMN

The national RDT algorithm in Zambia consists of a screening test (Determine® HIV 1/2) followed by confirmation of reactive specimens with a second rapid test (Uni-gold HIV 1/2). To test respondents via RDT, a blood sample is collected directly from a finger prick (ZDHS 2018).

According to the CDC, guidelines for HIV testing continue to evolve with changes in testing technology and methods to reach persons who can benefit from these services. The ministry of health Zambia recommends the testing algorithm shown below, which could include the following RDTs




## SCOPE OF THE STUDY

The study analyzed data collected under the STAR clinical performance study conducted by Zambart in the period of June 2016 to June 2017, therefore all analysis is limited to information collected during the STAR study period. This means that only the 5 HIV testing techniques that were included in the study will be analyzed. Neither the fourth generation RDT (Determine®HIV-1/2 Ag/Ab Combo rapid test) nor a Nucleic Acid Amplification Test, (NAAT-PCR) were included in the study due to financial reasons.

## STUDY OBJECTIVES

**MAIN OBJECTIVE**

The main aim of this study was to examine and compare five HIV testing techniques currently being used in Zambia by testing different samples of individuals in urban Mtendere and rural Kanakantampa. The results from this analysis will help give appropriate recommendations to the Ministry of health HIV testing algorithm.

**SPECIFIC OBJECTIVE**

To evaluate and assess the sensitivity and specificity of different HIV Tests used in Zambia: OraQuick ADVANCE Rapid HIV-1/2 Antibody Test, Abbot Determine™ HIV-1/2 antibody test, Uni-Gold™ Recombigen® HIV-1/2 rapid test, ARCHITECT HIV Ag/Ab Combo and the Genscreen™ ULTRA HIV Ag-Ab.

## METHODS

The study used quantitative methods by measuring statistical analysis of different variables of the five different HIV tests by using probability methods.

The study analyzed data collected from participants of M'tendere, Lusaka district and of Kanakantapa, Chongwe district (Ayles 2016). The table below shows the total number of participants who took part in the study from each site (Ayles 2016).

The study sample included all participants aged 15 years and over, living in the selected sites of Lusaka and gave informed consent to be part of the STAR study by Zambart (Ayles 2016).

**Frequency of the sites where the participants lived.**

**The two sites of the Participant**

| | | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Valid | Mtendere (Urban) | 1955 | 76.2 | 76.2 | 76.2 |
| | Kanakantampa (Rural) | 609 | 23.8 | 23.8 | 100.0 |
| | Total | 2564 | 100.0 | 100.0 | |

**STUDY POPULAION**

"Study Population" (also referred to as target group) refers to a group of subjects that contains features of interest to the researcher that meets specified characters pre-determined by the researcher (Polga & Thomas 2013; Jacobsen 2016). The study population comprised of all individuals in Lusaka that had an HIV test using: OraQuick ADVANCE Rapid HIV-1/2 Antibody Test, Abbot Determine™ HIV-1/2 antibody test, Uni-Gold™ Recombigen® HIV-1/2 rapid test, Abbot Architect HIV Ag/Ab Combo and the Genscreen™ ULTRA HIV Ag-Ab. This population was selected because of the proximity to the research Laboratory and because it included both rural and urban population.

## RESULTS

The specimen was collected by trained personnel following participant informed consent and complete assessment. Samples analyzed included oral mucosa transudate using the OraQuick® HIV Self-Test, fingerprick blood was collected and tested on Determine™ HIV1/2 (Alere), if positive on Determine it was then confirmed on Unigold™HIV1/2 (Trinity Biotech) test following the Zambian national testing algorithm. The results were provided to participants, and data stored in electronic devices, all participants with reactive test results were referred for HIV care and treatment at local health facility (Ayles 2016).

When all 3 rapid tests were compared to Abbot Architect, we see that OraQuick gives 22(0.86%) false negatives, UniGold gives 20(0.78%) false negatives and Deterimine had 18(0.70%) false negatives and 4(0.2%) false positives. Of the 3 RDTs, Determine is the most accurate with the highest Sensitivity (93.3%), then UniGold with 92.5% and lastly OraQuick with 91.7% sensitivity, which show a similar result pattern to a study by Kashoshi.

Plasma of the 2564 participants was tested on the Abbot Architect analyzer, 2297 (89.6%) were negative and 267 (10.4%) were positive as shown in the table 5. 2,311 (90.5%) had both a negative OraQuick and Determine test result. 245 (91.8%) participants had both a positive Determine and OraQuick test result. The sensitivity and specificity of the OraQuick test were 91.7 (95%CI) and 100 (95%CI), respectively when compared to HIV positive ABBOT test. The Sensitivity and Specificity of Determine test were 93.3% and 99.8% respectively.

**Gender, Age and HIV results compared to study sites of the participants**

| | Mtendere | | Kanakantampa | | Total | |
|---|---|---|---|---|---|---|
| **Characteristics** | n = 1955 | % | n = 609 | % | n = 2564 | % |
| **Gender** | | | | | | |
| **Male** | 719 | 28.0% | 324 | 12.6% | 1043 | 40.7% |
| **Female** | 1236 | 48.2% | 285 | 11.1% | 1521 | 59.3% |
| **Total** | 1955 | 76.2% | 609 | 23.8% | 2564 | 100.0% |
| **Age Groups** | | | | | | |
| **15 - 17** | 78 | 3.0% | 30 | 1.2% | 108 | 4.2% |
| **18 - 25** | 962 | 37.5% | 189 | 7.4% | 1151 | 44.9% |
| **26 - 35** | 554 | 21.6% | 149 | 5.8% | 703 | 27.4% |
| **36 - 45** | 228 | 8.9% | 110 | 4.3% | 338 | 13.2% |
| **46 - 55** | 78 | 3.0% | 55 | 2.1% | 133 | 5.2% |
| **56 - 65** | 41 | 1.6% | 35 | 1.4% | 76 | 3.0% |
| **66 - 75** | 10 | 0.4% | 28 | 1.1% | 38 | 1.5% |
| **76 - 85** | 4 | 0.2% | 9 | 0.4% | 13 | 0.5% |
| **86 - 95** | 0 | 0.0% | 4 | 0.2% | 4 | 0.2% |
| **Total** | 1955 | 76.2% | 609 | 23.8% | 2564 | 100.0% |
| **HIV result** | | | | | | |
| **Non-Reactive** | 1737 | 67.7% | 560 | 21.8% | 2297 | 89.6% |
| **Reactive** | 218 | 8.5% | 49 | 1.9% | 267 | 10.4% |
| **Total** | 1955 | 76.2% | 609 | 23.8% | 2564 | 100.0% |

According the table above, more female (63.2%) than male (36.8%) participants in Mtenedere took part in the study, while in Kanakantampa more male (53.2%) participants took part in the study compared to females (46.8%). In both sites the highest participating ages range from 18 to 45 years of Age.

**Gender and Age of Participants compared to ABBOT Results**

| | Abbot Architect Results (N) | | | | | |
|---|---|---|---|---|---|---|
| | Non-Reactive | | Reactive | | Total | |
| **Gender of Participant** | (N) | % | (N) | % | (N) | % |
| **Male** | 960 | 37.4% | 83 | 3.24% | 1043 | 40.7% |
| **Female** | 1337 | 52.1% | 184 | 7.18% | 1521 | 59.3% |
| **Total** | 2297 | 89.6% | 267 | 10.41% | 2564 | 100.0% |
| **Age Groups** | | | | | | |
| **15 -17** | 102 | 4.0% | 6 | 0.23% | 108 | 4.2% |
| **18 -25** | 1087 | 42.4% | 64 | 2.50% | 1151 | 44.9% |
| **26 - 35** | 599 | 23.4% | 104 | 4.06% | 703 | 27.4% |
| **36 - 45** | 275 | 10.7% | 63 | 2.46% | 338 | 13.2% |
| **46 - 55** | 111 | 4.3% | 22 | 0.86% | 133 | 5.2% |
| **56 - 65** | 71 | 2.8% | 5 | 0.20% | 76 | 3.0% |
| **66 - 75** | 36 | 1.4% | 2 | 0.08% | 38 | 1.5% |
| **76 - 85** | 12 | 0.5% | 1 | 0.04% | 13 | 0.5% |
| **86 - 95** | 4 | 0.2% | 0 | 0.00% | 4 | 0.2% |
| **Total** | 2297 | 89.6% | 267 | 10.41% | 2564 | 100.0% |

The comparison between these diagnostic tests forms the foundation of this comparative evaluation of HIV testing techniques. Serologic test results were divided into Reactive and Non-Reactive findings, as recommend by the manufacturers of the diagnostic tests. The table above compares gender and Age of participants to HIV test results of the Abbot, used as a reference. As expected, female participants (68.9%) had a higher positivity rate compared to male participants (31.1%). The age range with the highest positive results was 26 to 35 years.

**Results of the 5 HIV tests being investigated**

| | Ora-quick | | Determine | | Uni-Gold | | Abbot Architect | | BIORAD-ELISA | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | % | N | % | N | % | N | % | N | % |
| **Non-Reactive** | 2319 | 90.4 | 2311 | 90.1 | 6 | 0.2 | 2297 | 89.6 | 1 | 0.0004 |
| **Reactive** | 245 | 9.6 | 253 | 9.9 | 247 | 9.6 | 267 | 10.4 | 266 | 10.4 |
| **Not Tested** | 0 | 0 | 0 | 0 | 2311 | 90.1 | 0 | 0 | 2297 | 89.6 |
| **Total** | 2564 | 100 | 2564 | 100 | 2564 | 100 | 2564 | 100 | 2564 | 100 |

**ORA-QUICK:** Of the 2564 samples tested on this test, 2319 were non-Reactive and 245 were Reactive. All samples had a result.

**DETERMINE:** all samples tested on Ora-quick were tested on Determine, out of the 2564 samples tested, 2311 were non-Reactive and 253 were Reactive.

**UNI-GOLD:** Since this was used as a confirmatory test, only samples Reactive on the Determine test were tested on Uni-Gold. 253 samples Reactive on Determine were tested on Uni-gold, 247 were Reactive and 6 samples were non-Reactive. 2311 were not tested because they were Non-Reactive on Determine.

**ABBOT ARCHITECT:** The Abbot Architect was the gold standard test were all above tests were compared to. All 2564 samples were tested on this test, 2297 samples were non-Reactive, and 267 samples were Reactive.

**GENSCREEN ULTRA ELISA:** This was a confirmatory test for the Abbot Architect test. Only samples Reactive on Abbot were tested on this test. Of the 267 samples tested, 266 were Reactive and 1 which had a low signal to cut off on Abbot Architect tested Negative. This 1 discordant result was tested on Geenius confirmatory test, and it was Non-Reactive, showing that the Genscreen ultra ELISA is slightly more sensitive than the Abbot Architect test.

## DISCUSSION

The study established that the 3 3rd generation HIV RDTs used at Point of Care for HIV testing though quite sensitive, still fail to detect a significant number of acute infections. About 1 in every 10 people tested using 3rd generation RDTs goes home with false negative results. The identification of persons with acute HIV infection represents a significant challenge, owing to the absence of antibodies in the earliest stages, limitations of standard rapid tests to detect p24 or HIV RNA, and logistical and cost issues with p24 antigen and HIV RNA. In resource-limited or point-of-care settings, rapid diagnostic tests (RDTs), that aim to simultaneously detect HIV antibodies and p24 capsid (p24CA) antigen with high sensitivity, can pose important alternatives to screen for early infections.

The study also 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. During this period, HIV infection can be detected only by tests detecting also viral antigen such as the laboratory-based 4th generation ELISA.[22] The baseline HIV prevalence in the population influences the negative and PPV and needs to be considered in the interpretation of HIV RDT results. On the other hand, it is not certain that the currently available 4th generation HIV RDTs would perform better based on recent published data showing that the HIV p24 antigen detection component of some 4th generation RDTs also lacks analytical and diagnostic sensitivity.

## CONCLUSION

This observation raises the possibility of unacceptably low sensitivities of the RDTs Alere Determine HIV-1/2 and Uni-Gold HIV, using blood and, OraQuick ADVANCE® Rapid HIV-1/2 using mouth swabs in Zambia. This raises serious concerns about rapid test dependability and calls for stringent control measures as well as the need for 4th generation Ag-Ab RDTs and a cross-evaluation of all Rapid diagnostic tests results by a laboratory-based antigen testing, whenever feasible. The results of this study have several important clinical and public health implications, for example 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) (Kashosi TM et al, 2018).

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