Research Article

Comparison between the accuracy of a rapid point-of-care HIV test TOYO versus ELISA, Western Blot, and RT-PCR analysis

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Abstract

Background: The human immunodeficiency virus (HIV) is considered one of the most serious health challenges worldwide. Early detection and diagnosis are key elements in the effort to control HIV. Rapid tests for the detection of HIV-antibody allow timely point-of-care provision of results and do not require the laboratory facilities needed for conventional enzyme immunoassays (enzyme-linked immunosorbent assay [ELISA]) and Western blot (WB) testing. Regardless of the advantages of these tests, other studies showed that the sensitivity of the performance of some rapid test brands comparing with the lab standard methods have some limitation.

Objective: This study evaluated and compared the performance of commercially available HIV rapid test TOYO against the gold standard tests used in the clinical laboratories at Saudi Arabia.

Methods: Forty-two male and female patient samples were tested with real-time polymerase chain reaction (RT-PCR), ELISA, and/or WB followed by TOYO rapid test to compare their results when performed under similar conditions.

Results: The higher compatibility of TOYO results was observed with RT-PCR with 81% followed by WB and ELISA with 76% and 63%, respectively. However, more than 25% of the results showed either false positive or false negative results when compared with either of the standard diagnostic tests used in this study.

Conclusion: This study have provided evidence that TOYO rapid HIV assay cannot perform equally as RT-PCR, ELISA, or WB for the detection of HIV antibodies. A further study is suggested to compare the performance of TOYO rapid test against standard diagnostic tests with larger number of patients' samples.

KEY WORDS: Rapid test, TOYO, human immunodeficiency virus

Introduction

The human immunodeficiency virus (HIV) was isolated in 1983–1984 and considered to be one of the most serious health challenges worldwide.[1] Despite the progress made toward prevention and treatment, virtually no country in the world remains unaffected, and the Kingdom of Saudi Arabia is no exception.[2] In 1984, the first case of HIV was diagnosed in the kingdom.[3] Since then, information about HIV cases from different regions in the Kingdom began arriving at the Ministry of Health.

Early detection of anti-HIV antibodies as a marker of HIV and accurate diagnosis of the infection are crucial factors for timely identification of affected patients, prevention strategies, and control of the disease.[4,5] Early diagnosis of HIV has a number of advantages by allowing infected individuals to access anti-HIV treatment before their immune systems have been severely damaged, which can also improve their survival and long-term health outcomes. New HIV infections might be

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also prevented with early detection. Several studies revealed that newly infected persons who become aware of their status can make more informed decisions (such as practicing safer sex) and take steps to prevent HIV transmission.[6–8] Thus, efficient and accurate HIV testing programs is urgently needed to provide a quick identification for the disease.

The current standard methods for the diagnosis of HIV in Saudi Arabia are an initial test using enzyme-linked immunosorbent assay (ELISA) and a confirmatory test using Western blot (WB).[3] In addition, these investigatory methods have been considered as golden standard techniques for HIV detection in many other countries including the United States,[9] Canada,[10] and Brazil.[11] However, these standard techniques require expensive instrumentation, technical expertise, and labor-intensive and time-consuming format of the assay.[5,9]

Rapid point-of-care tests can play vital role in this scenario.[12] The availability, safety, and the cost–effectiveness with the time-consuming and ease of use of these tests are the valuable reasons to use them for patients' investigations.[4] Moodley et al . (2008) found that HIV rapid tests have increased the clients' confidence because the tests are performed in their presence and the chance of errors with incorrectly labeled specimens is minimal.[13]

Regardless of the advantages of these tests, the reliabilities and the sensitivities of the performance of some commercial rapid test brands comparing with the lab standard methods have some limitations.[14] A number of studies have raised concerns regarding the accuracy and sensitivity of these tests.[5,10,15] Different considerations have been taken into account; one of these could be related to the various concentrations of viral loads in patients' samples as the low viral load cannot be detected in some cases by the rapid tests.[16] In 2010, Wolpaw and colleagues reported low levels of rapid HIV test sensitivity, when performed on patients with low levels of HIV antibody.[15] Similarly, the sensitivity for some HIV rapid test kits was as low as 92%, suggesting that not all rapid tests perform optimally.[17] Another study has advised the patient who received a negative result of rapid HIV test to be re-tested using the conventional standard lab methods to be confirmed.[16] Moreover, a different study, reported false positive results in very few cases depending on the types of the used rapid HIV tests.[14]

On the other hand, high sensitivity (greater than 99%) and specificity (greater than 98%) levels were reported in Canada for two licensed point-of-care HIV tests including; Genie HIV-1/2 and Fast-Check HIV-1/2.[4] The World Health Organization (WHO) has also acknowledged the need for measuring the accuracy of the performance of rapid HIV testing and comparing their results with the conventional standard methods before implementation of the kit.[18]

Reliability and sensitivity of four HIV rapid tests were compared against the ELISA and showed a sensitivity level ranged from 92.5% to 97.3% and 97.6% to 98.2%, respectively, when performed by nurses.[13] The result interpretation, the quality assurance, and the training of operators of rapid HIV tests play major part in the accuracy and the sensitivity of results.[19]

The aim of this study is to assess the accuracy of rapid point-of-care HIV blood test TOYO DIAGNOSTIC (LOT NO.HIV021401) that is one of the provided kits by the Ministry of Health for a number of clinical centers in Makkah, and compare its performance with three conventional standard methods: ELISA, WB, and real-time polymerase chain reaction (RT-PCR). Moreover, this study evaluates the validity of rapid tests subsequently. If the TOYO rapid HIV test showed a high sensitivity, it can be used within the government clinical laboratories of the Ministry of Health hospitals. The correlation between the performance and the assay principles might be different but the results should be moderately comparable and that what the purpose of this research is attain to realize and investigate. We believe that since results of rapid tests can be available immediately, the tests can be used easily and efficiently in most emergency situations.

Materials and Methods

This study was conducted in a regional laboratory in Saudi Arabia where the samples were received from different hospital laboratories in order to be investigated. Samples were collected from 42 male and female patients over the period of December 2014 to February 2015. Thus, all the received samples were either confirmed positive or confirmed negative based on ELISA and/or WB. Whole blood samples were collected from suspected patients into EDTA tubes (VACUETTE® K3 EDTA Tube, USA), followed by centrifugation at 4,000 rpm (Heraeus® Labofuge® 200, USA) for 10 min, then plasma was separated and transferred to plan tubes with no additive using sterile filter tips to be investigated by PCR and TOYO rapid test.

Ribonucleic acid (RNA) extraction was done in order to be examined by real-time PCR using automated M24sp (Abbott, USA). Extraction was done according to the manufacturer's instructions using the magnetic particles of RNA preparation kit (Abbott) to yield a target, which was used for amplification. RNA was extracted from 500 µL plasma and the elutant was 50 µL. Internal control (Abbott) was kept for each sample during the extraction process. Version 7.0 EVO software of M24sp (Abbott) of HIV RNA assay protocol was used for the extraction.

RT PCR amplification was performed in a 100-µL reaction mixture containing 50 µL of prepared PCR Master Mix and 50 µL of extracted RNA. This was added to 96-well PCR plate (Abbott) and properly sealed to be carried out by M2000rt (Abbott) following the manufacturer's instructions using HIV amplification kit (Abbott).

TOYO rapid test (TOYO LOT NO.HIV 021401), which has 100% sensitivity and specificity according to the manufacturer's insert sheet, was used as a rapid qualitative immunoassay for the detection of anti-HIV 1/2/O in plasma. Fifty microliter of plasma was added to the test cassette devices avoiding formation of air bubbles. Results were obtained within 15 min depending on the anti-HIV concentration in each sample. The visibility of the color on the cassette device was

an indication for the existence of HIV-antibody. Internal procedural control was considered with each sample. The results were evaluated visually.

Statistical Analysis

Sample size was 42. The results were transferred to Excel spreadsheet and grouped by the compatibilities of each sample with the tests of our interest whether for RT-PCR vs.TOYO rapid test, ELISA vs.TOYO rapid test, and WB vs.TOYO rapid test. Data were analyzed using Microsoft Excel to calculate the frequencies as percentages. The calculation was made including the positive and negative results. However, all weak positive results of ELISA and WB were excluded from the study.

Ethics Committee Approval

Verbal approval was obtained from the director of administration laboratory and blood banks in Holy Makkah before the researchers establish the study.

Results

An evaluation of a commercial HIV TOYO rapid test was done to compare its performance against standard tests used at the Ministry of Health medical centers and hospitals at Makkah. A total of 42 male and female samples were screened with standard investigations RT-PCR and of those 21 tested with WB followed by TOYO rapid test evaluation, whereas 30 samples were verified by ELISA then examined by TOYO rapid test. The results of TOYO rapid test were interpreted visually (Figure 1). The compatibility of TOYO rapid test results versus the results of RT-PCR in a total of 42 tests was 81%, which indicates that the accuracy of TOYO rapid tests with RT-PCR was high when compared with the other investigated assays (Figures 2–4). On the other hand, noncompatibility was also seen between HIV rapid test and RT-PCR with 19% of samples (Figure 2). The results from this study reported a lower compatibility of TOYO rapid tests versus the results obtained with ELISA assay as it showed 63% and 37% compatible and noncompatible results, respectively (Figure 3). Meanwhile the WB results showed much more

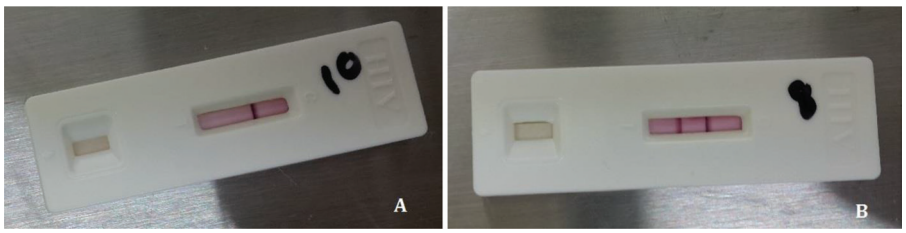

Figure 1: Interpretations of HIV results from TOYO rapid test; 50 µL plasma sample was added to the well of the test device without any dilution. The result was visually read within 15 min. Only one red line, which is a test control line, was observed in a negative sample (A) while for the positive sample, two red lines were developed (B).

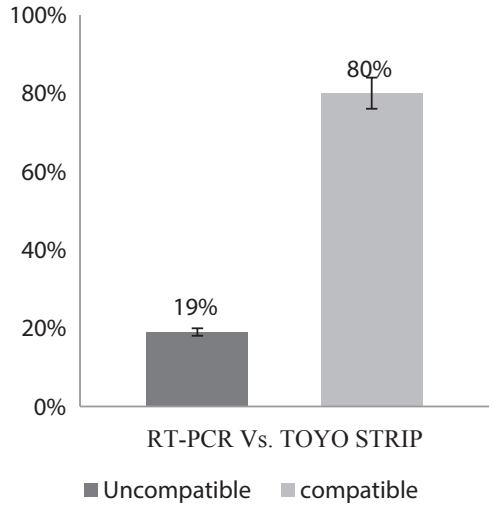

Figure 2: Percentage of the compatibility of RT-PCR-with Toyo test in order to compare the accuracy of RT-PCR versus Toyo test. These results represent the percentage values including error bars of independent investigations.

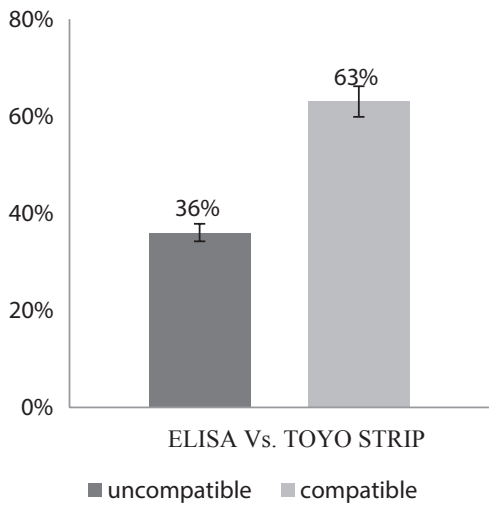

Figure 3: Percentage of the compatibility of ELISA-with Toyo test. These results represent the percentage values including error bars of independent investigations.

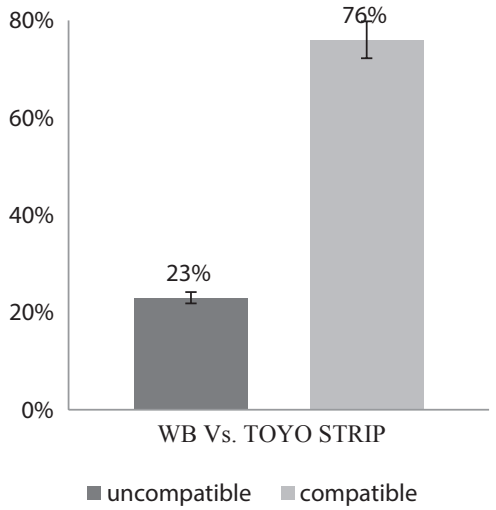

Figure 4: Percentage of the compatibility of WB with Toyo test. These results represent the percentage values including error bars of independent investigations.

compatibility with TOYO rapid test results than ELISA by 76% in a total of 21 samples (Figure 4).

The variation of different assay results could be related to the sensitivity and the specificity of each assay. The interpretations of TOYO results with that of the RT-PCR assay were the most accurate, regardless of the sample size. The percentage of the accuracy of TOYO test was obviously well-matched with the majority of the examined samples by RT-PCR under the same conditions, which were used with the other assays. However, the compatibility of the results from TOYO test showed the greatest variation with ELISA than others (Figure 3). No invalid or indeterminate test results were noticed while using the Toyo test devices.

Discussion

Accurate diagnosis and early detection of HIV infection is essential for timely identification of patients needing antiretroviral treatment and for instituting HIV prevention strategies. Rapid antibody tests for the detection of HIV offer an effective means of providing rapid results outside the laboratory, which minimize the need for expensive equipment and provide a quick investigation with minimum cost, time, and effort. Manufacturers of these commercial rapid HIV tests reported 95%–100% specificity and sensitivity; however, these need further investigations and comparison particularly with the standard ELISA and WB tests, which are commonly used to detect the HIV-antibody.[9] In addition, the WHO has acknowledged the need for assessing the performance of rapid HIV testing, personnel who perform the tests, site visits to observe testing, and external quality assurance based on retesting a proportion of specimens by reference laboratories before implementation of the HIV rapid kit.[18] Thus, the need for assessing the accuracy and sensitivity of rapid HIV assays is highlighted nowadays.

This study is the first attempt to evaluate systematically the commercially available TOYO rapid test in the laboratory of government hospitals and medical centers at Makkah and compare its performance with the gold standard testing used for HIV diagnosis.

The majority of investigated samples of patients (more than 80%) showed similar results when tested with TOYO rapid test and RT-PCR assay when performed under similar conditions (at the same lab and by same laboratory personnel). There is evidence, however, that rapid HIV test kits were unable to detect the infection, which was PCR positive and was classified as acute HIV infection.[15]

Among 30 samples were investigated by ELISA followed by TOYO rapid test showed less than 63% were given the same results. In 2008, Moodley et al. compared the performance of four commercial HIV rapid tests (First Response HIV Card Test 1-2.0, Pareekshak HIV Triline, Abbott-DetermineTM HIV-1/2, and SENSA) and reported a high level of sensitivity and specificity when compared with their corresponding ELISA results when performed by nurses and laboratory technicians.[13] Within the same study, they found a significant difference at the level of the sensitivity between different HIV rapid tests.[13] A more recent study showed low sensitivity level and false positive results for HIV rapid diagnostic tests when compared with ELISA.[5]

This study also compared the performance of TOYO rapid test with WB and showed a higher competency rate than that with ELISA. However, more than 20% of investigated samples did not show compatible results when tested with TOYO HIV rapid test and WB. One possible reason for these differences between the results obtained with TOYO HIV rapid test and RT-PCR, ELISA, or WB may be due to low antibody titers, especially in patients with recent infections where the levels might be below the detection limit of TOYO test.

This study was the first to compare the performance of TOYO HIV rapid test in the Kingdom of Saudi Arabia. Based on our findings, we suggest that the standard diagnostic testing should be employed in all the clinical laboratories since TOYO has been found to give some false negative and false positive results when compared with RT-PCR, ELISA, and WB. Also, while the manufacturer reported 100% sensitivity for TOYO HIV rapid test, we found differences among some patient's results; thus, further studies should be done to compare the results between TOYO and the standard HIV detection methods with larger number of patients' samples.

Conclusion

This study has provided evidence that TOYO rapid HIV assay cannot perform equally as RT-PCR, ELISA, or WB for the detection of HIV antibodies. Further study is suggested to compare the performance of TOYO rapid test against standard diagnostic tests with larger number of patients' samples.

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