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A comparative study on the use of Determine rapid test kit buffer and substitute liquids in HIV Rapid testing

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Abstract

Live HIV rapid test kits (RTKs) are usually packaged with only a single test buffer vial. A major challenge with HIV testing is the problem of lack of the RTK buffer vial to complete the number of tests in an RTK pack due to misuse, misplacement or contamination of buffer with clients' blood during HIV testing. This has led to the use of sterile normal saline and tap water as substitute liquids in place of the buffer vial. This study compared the use of the commercially provided buffer vial and these substitute liquids in HIV rapid testing while using the commercially provided buffer as gold standard. One hundred (100) HIV positive samples from patients attending antiretroviral therapy (ART) clinic and 100 HIV negative blood samples from blood donors placed in EDTA were tested with Determine RTK using the commercially provided buffer and substitute liquids (Sterile normal saline and tap water). The Determine RTK was also tested using the commercially provided buffer, normal saline and tap water without addition of blood samples. Results of the tests with substitute liquids were compared with those obtained with that of RTK's commercially provided buffer. With HIV positive and negative blood samples, the expected test line and control line or only control line (for negative samples) were observed with the commercially provided buffer and sterile normal saline. When tested without blood samples using the RTK's buffer and normal saline, the control line was observed but no test line for all 100 tests done. Invalid test results were recorded in 48% of the negative samples and 40% of test with no blood added when with tap water was used. This study also shows varied colour intensity for the buffer vial and substitute liquids used. For samples tested with sterile normal saline, 52% of the tested samples showed stronger colour intensity in test line that control line, 37% had equal colour intensity while 11% had higher colour intensity in control than test line. Seventy seven (77%) of Samples tested with tap water had the colour intensity greater in test line than control line while 23% had equal colour intensity in both test and control line. With sterile normal saline used with HIV positive and negative blood samples, a 100% clear background like that of the gold standard was obtained. However, tap water when used as a substitute buffer to run all 100 HIV positive samples had an unclear background. An unclear background was also observed in 100% and 28% of the test ran with tap water only (without blood samples) and with HIV Negative samples respectively.

Keywords

Determine,
Rapid test kit,
Buffer,
Substitute liquids

There were no false positive and false negative result with any of the substitute liquids when tested with HIV negative blood, HIV positive blood and without blood samples. Sterile normal saline gave similar results as the commercially provided buffer but tap water gave invalid results when used with HIV negative blood samples and without blood samples. This study shows that the use of sterile normal saline as substitute buffer is comparable to commercially buffer vial in testing of HIV with Determine RTK.

Introduction

Nigeria is currently implementing HIV rapid testing in health facilities and community outreaches using the multi-points testing model. The objective is to enhance universal access to prevention, care and treatment of HIV infection through real-time testing and result collection (World Health Organization, 2015). This multi-points testing model has resulted in increased number of rapid HIV tests being done by non-laboratory personnel such as lay counsellors and nurses. With minimum training and without the requirement for laboratory facilities or expensive equipment, these non-laboratorians perform tests in addition to counselling. However, ensuring the quality of HIV testing at several testing sites including unconventional testing settings have continued to be a major challenge (Department of Health and Human Services Centers for Disease Control and Prevention; Heneke, 2009; Sanjana et al, 2009; Klarkowski et al, 2014; Mwangala et al, 2015)

Determine HIV rapid test kit (RTK) detect antibodies against HIV virus in blood by an antibody-antigen reaction on a nitrocellulose strip. Reactions are shown as visible as pink/red lines to display a control line and a test result line (Determine User's manual).

HIV RTKs approved for use in Nigeria by the Federal Ministry of Health in the National Testing Algorithm are marketed either as kits or cassettes that include material for either 20 or 100 tests, just like the test strips/cassettes themselves, lancets for finger pricking, transfer devices (capillaries tubes) and the buffer (FMOH, 2010). The strips/cassettes are usually individually packaged with the number of lancets and transfer devices matching the number of strips/cassettes. All HIV RTKs need a buffer for use with whole blood to allow capillary flow along the nitrocellulose strip or cassettes. Mostly, this buffer is supplied (commercially provided) in a single dropper vial (Determine User's manual).

Problem Statement

One of the major challenges with HIV rapid testing by non-laboratorians and some laboratorians is the problem of lack of the commercially provided buffer vial to complete the test in an RTK pack. This could be due to misuse, misplacement of the buffer or contamination of buffer with clients' blood during HIV testing at testing points. On-the-ground experience in real-life situations, even in laboratory settings, proves that more commercially provided (chase) buffer is often needed than is provided by the manufacturer. To compensate for this, health workers involved in HIV testing either use a buffer vial from another pack (sometimes from a kit of another brand) or normal saline, distilled water used for the dilution of drugs and occasionally tap water as substitute liquids. The substitution of kits' buffer seems not to cause much interference with the reaction, as the kits seem to have good background clearance and both control line and test lines after test are clearly distinguishable (Gillet, Mori, Van den Ende & Jacobs, 2010). This study, therefore, seeks to compare the use of the commercially provided buffer and some substitute liquids in HIV rapid testing.

Objectives of the Study

1. To compare the use of commercially provided HIV testing buffer and substitute liquids in HIV testing with Determine RTK;
2. To compare the colour intensity of the control and test lines of Determine RTK with commercially provided buffer and substitute liquids in HIV testing;
3. To compare the clearance of the background of test strip with use of commercially provided buffer and substitute liquids in HIV testing with Determine RTK;
4. To assess the effect of buffer substitution on Determine RTK test results.

Methods

EDTA-blood samples collected from one hundred (100) HIV negative blood donors and 100 clients attending HIV clinic for ART were used for this study. The samples were de-identified and 6 categories of testing were done with Determine rapid test kit. Group A testing was done on all the 200 blood samples with

Determine using commercially provided buffer; group B, all 200 blood samples with Determine using normal saline solution and group C all 200 blood samples with Determine using tap water from local source while group D was 100 test done with Determine and commercially provided buffer without blood samples added; group E, 100 test with Determine and normal saline without blood samples added and group F 100 test with Determine and tap water but without blood samples added.

The RTKs kits used for this study were of the same Lot number and expiry date and were used within their expiry date and were stored at 2^{oc} to 30^{oc} according to manufacturer's instruction prior to being used for the analysis. The RTKs were subjected to internal quality control testing with both positive and negative dried tube specimen (DTS) panels before running with the participants' blood using commercially provided buffer and each of the substitute liquids. Ditto also for tests ran without blood samples added, with the commercially provided buffer or substitute liquid only. All tests were performed according to manufacturer's instruction. Readings were performed by three readers at daylight after the prescribed 15 minutes' incubation period after application of the blood sample and buffer, blood sample and substitute liquids or only buffer or substitute liquids. Where the control line did not appear, the result was considered as invalid and the test was repeated with another test strip. RTK test lines were interpreted according to the manufacturers' instructions. In addition, the colour intensities of the test line of the kits were grouped into five categories: 1 (no line visible), 2 (barely visible line), 3 (paler than the control line), 4 (equal to the control line) or 5 (stronger than the control line). Observers were blinded to each other's' reading. The results of the readings considered were based on consensus agreement (Van der Palen et al, 2009). The colour and intensity of the control and test lines and the clearance of the background (whether clear or cloudy) when substitute liquids were used were compared with those obtained with the commercially provided buffer. The results obtained with the substitute liquid with and without blood (positive and negative) were compared to those obtained with the commercially provided buffer with both negative and positive samples.

Alere DetermineTM HIV-1/2 Test, a single-use immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2. The sample (whole blood) is added to the sample pad followed by Chase Buffer. As the sample and buffer migrates through the conjugate pad, it reconstitutes and mixes with the antigen conjugate. The mixture migrates to the immobilized recombinant antigens and synthetic peptides which are present at the patient window site. If antibodies to HIV-1 and/or HIV-2 are present in the sample, the antibodies bind to the antigen-selenium colloid and to the antigen at the patient window, forming a pink/red line at the patient window site. If antibodies to HIV-1/2 are not present, the antigen-selenium colloid flows past the patient window and no pink/red line is formed at the patient window site. A procedural "Control" line containing HIV-1 p24 recombinant antigen incorporated in the nitrocellulose membrane ensures assay validity. For a test result to be valid there must be a visible pink/red line at the control area of the kit (Alere Determine User's manual).

Ethical Clearance

The study was approved by the Ethical Committee of Bayelsa State Ministry of Health.

Results

Characteristics of the Study participants

The participants were 200 made up of 109 females (54.5%) and 91 males (45.5%). The 200 samples were made up of 100 positive samples (63 females and 37 males) and 100 negative samples (54 males and 46 females). The ages of the participant ranged from 2 to 72 years.

Use of commercially provided HIV testing buffer and substitute liquids in HIV testing

Test results came out within the 15minutes time period specified by the kit manufacturers with the commercially provided and substitute buffers when used to test both HIV positive and negative samples.

When HIV positive blood samples were tested with Determine strips and the RTK kit's chase buffer, the expected test line and control results were observed in all 100 samples. The expected test line and control results were also observed when HIV positive blood samples were tested with Determine strips and substitute liquids (sterile normal saline and tap water) used in place of the kit's chase buffer in all 100 samples. Similarly, when HIV negative blood samples were tested with Determine strips and the RTK kit's chase buffer, the expected control line was observed but no test line appeared in all 100 samples. Same result was obtained when HIV negative blood samples

were tested with Determine strips with sterile normal saline used as substitute buffer. However, invalid test results (i.e. no visible test and control lines observed) were recorded in 40 (40%) of the test done when tap water was used as buffer with HIV negative blood samples (Table 1).

When the RTK was tested without blood samples using the commercially provided chase buffer and normal saline as substitute buffer, the control line was observed but no test line for all 100 tests done with each liquid. However, invalid test results were recorded in 48 (48%) of the test done when tap water was used as substitute buffer without adding blood samples (Table 2).

Table 1: No. of positive and negative results with commercially provided chase buffer and substitute liquids with blood sample

Categories	Positive result	Negative Result	Invalid result	Sensitivity	Specificity
Chase Buffer	100 (100)	100 (100)	0 (0)	100	100
Normal Saline	100 (100)	100 (100)	0 (0)	100	100
Tap water	100 (100)	60 (60)	40 (40)	60	60

Table 2: No. of invalid results with commercially provided chase buffer and substitute liquids with and without blood sample

Categories	Chase Buffer	Normal Saline	Tap Water	Total
Positive Sample	0 (0)	0 (0)	0 (0)	0
Negative Sample	0 (0)	0 (0)	40 (40)	40
Without Sample	0 (0)	0 (0)	48 (48)	48

Table 3: No. of negative results with commercially provided chase buffer and substitute liquids without blood sample

Categories	Negative Result	Invalid result
Chase Buffer	100 (100)	0 (0)
Normal Saline	100 (100)	0 (0)
Tap water	52 (52)	48 (48)

Test line colour intensity for the RTK when run with HIV Positive Blood Samples Using Determine Chase Buffer and Substitute Liquids

The intensity of the colour of the test line of the RTK was between 3 and 5 when the commercially provided buffer was used with HIV positive blood samples. Those that had colour intensity of 4 and 5 were both 40% and 42% of the samples respectively while 18% had intensity of 3. For tap water, the test line colour intensities were 5 (69%), 4 (29%) and 3 (2%). The colour intensities when sterile normal saline was used were 5 (56%), 4 (31%) and 3 (13%) Table 4).

Table 4: Test line colour intensity of the RTK when run with HIV Positive Blood Samples

Buffers	3	4	5	Total
Chase Buffer	18 (18)	40 (40)	42 (42)	100
Normal Saline	13 (13)	31 (31)	56 (56)	100
Tap water	2 (2)	29 (29)	69 (69)	100

where 1= none (no line visible); 2= faint (barely visible line); 3= weak (paler than the control line); 4= medium (equal to the control line) and 5 = strong (stronger than the control line)

Clearance of the background of the test strip when run with HIV Positive and Negative Blood Samples Using Determine Chase Buffer and Substitute Liquids

Sterile normal saline as the substitute liquid when used with HIV positive and negative blood samples, the RTK showed a clearance of background similar to those observed with the RTK commercial buffer (100%). The background of the test strips was not clear when tap water was used as a substitute buffer to run all 100 HIV positive samples. However, the background was clear in 100% and 28% of the test ran with tap water without blood samples and with HIV Negative samples respectively.

HIV Test Results for Determine RTK with Substitute Liquids

There were no false positive and false negative result with any of the substitute liquids when tested with HIV negative and positive blood samples and without blood samples. When tested with tap water in the absence of blood, 48 (48%) of the test gave invalid results. Also, 40 (40%) of the test with tap water and HIV negative blood gave invalid results (Table 2).

Discussion

This study shows that replacing Determine RTK commercially provided buffer with sterile normal saline and tap water did not give false positive or negative results and that the control and test lines observed with sterile normal saline were similar to those obtained with Determine commercially provided buffer. This finding is contrary to findings by Gillet et al, (2010) in a study on buffer substitution for malaria rapid diagnostic tests (RDTs). However, there were cases of invalid test results with tap water when used as buffer to test HIV negative blood samples and run the test without blood samples. This agrees with the findings by Gillet et al, (2010) in their study with the use od distilled water. There may be wastage of the RTK due to invalid results when tap water is used as buffer in HIV testing with Determine and this in turn will lead to increase in cost of HIV testing.

Also, the clearance of background of the RTK was similar to those with the RTK commercially provided buffer when normal saline was used as substitute liquid with HIV positive and negative blood samples. However, the background of the test strips was not clear when tap water was used as a substitute buffer to run HIV positive samples but clear in 100% and 28% of the test ran with tap water without blood samples and with HIV Negative samples respectively. These findings are contrary to those by Gillet et al, (2010) where tap water gave a clearance of background similar to those observed with the RDT kit's buffer while normal saline gave a less clearer background.

Furthermore, this study has shown that a control line will be visible as long as migration has been achieved irrespective of the liquid used. The buffer re-solubilizes polymers and proteins on the sample pad and the dried detection antibody-conjugate on the conjugate pad.

Conclusions

In conclusion, this study shows that buffer substitution with sterile normal saline and tap water in HIV testing with Determine RTK kit does not cause false positive or false negative test results. While commercially provided chase buffer is always preferred, we recommend that the sterile normal saline can be used as substitute liquids when commercially provided buffer vial is not available. This is to ensure that HIV testing for those seeking the test is not delayed. However, periodic quality control should be carried out to further ensure the quality of the test results.

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