SHORT COMMUNICATION

The use of 0.01M phosphate buffered saline as detection buffer for Alere Determine® HIV rapid test in resource limited settings

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Abstract

Insufficient supply of manufacture's buffers/diluents in relation to the number of strips per kit has been found to have negative impact on patients' results. Some laboratories personnel tend to use diluents from other rapid tests manufacturers such as Bioline, Unigold as well as malaria rapid diagnostic test (MRDT). This study aimed at evaluating the use of 0.01M phosphate buffered saline (PBS) as detection buffer for Alere Determine® HIV rapid test. This study was carried out at Bugando School of Medicine in Mwanza, Tanzania. A total of 300 whole blood specimens; 150 HIV positive specimens from patients attending Care and Treatment Centreand 150 HIV negative specimens were retested for HIV status using Alere Determine® HIV rapid test employing normal Alere buffer and 0.01M PBS as buffer.Of the total specimens tested; 150 (100%) of HIV positive were positive by using both Alere buffer and 0.01M PBS while 150(100%) of HIV negative samples were negative by both Alere Determine® and 0.01M PBS. The agreement between 0.01M PBS and Alere Determine® buffer was 100%. The value of kappa indicates perfect agreement between 0.01M PBS and Alere Determine® buffer (100%). A 0.01M PBS is recommended as alternative detection buffer for Alere Determine® in cases of insufficient supply. Further investigation to evaluate the suitable buffer for other rapid tests for HIV and other diseases is recommended especially in resource limited settings.

Keywords: Phosphate buffered saline, Alere Determine, rapid test, HIV

Accurate HIV diagnostic testing is the key step to identify infected individuals for appropriate care. This is highly reliant on early and precise detection of antibodies to HIV1 and HIV2 in blood by rapid tests(NACP, 2012). Constant supply, availability of sufficient and accurate diagnostic materials as well as well qualified laboratory personnel facilitates the goal for HIV control. This also goes together with discouraging the habit of testing HIV in non- laboratory settings and by non-qualified personnel to avoid procedural errors that may lead to false positive and negative results(Johnson *et al.*, 2015).

In response to the call for universal access to prevention, care and treatment by United Nations AIDS (UNAIDS) and World Health Organization (WHO), HIV rapid testing has been expanded worldwide (Parekh *et al.*, 2010). However, HIV diagnosis is performed by personnel with varied skills and limited resources which poses challenges related to test selection, test kit quality, algorithms, quality assurance, insufficient volume of buffers and post market evaluation(Parekh et al., 2010, Johnson et al., 2015, Klarkowski et al., 2009).

The market for HIV diagnostics has expanded in recent years with the availability of a diverse number of rapid test kits. However, not all kits are manufactured with acceptable standards. Some kits are produced by the same manufacturer but sold under different names by different companies. Occasionally, the same kits or its components are manufactured in multiple facilities, some of which may lack approval by local and international regulatory authorities. In some occasions composition and specifications are not indicated by manufacturer. Other manufacturers have documented the use of PBS in the kits without further specifications (https://www.fda.gov/downloads/BiologicsBloodVaccines/BloodBlood-Products/ApprovedProducts/PremarketApprovalsPMAs/UCM092003.pdf).

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In resource limited setting the use of whole blood for HIV diagnosis is mostly used whilst the volume of buffer supplied with the kits are not sufficient most of the times. This results into the cross use of buffers from different rapid tests and invalid locally-made buffers. Phosphate buffered saline (PBS) is a common buffer used in biological research. It is a water based solution containing sodium chloride, sodium phosphate and in some formulations potassium chloride and phosphate with the main function of maintaining a constant pH. The aim of this study was to evaluate the usefulness of PBS as alternative HIV detection buffer.

This was a laboratory based study conducted between June and July 2013 at the Weill Bugando School of Medicine in Mwanza, Tanzania. A total of 300 blood specimens were collected whereby 150 were from HIV positive patients attending care and treatment centre (CTC) Bugando and 150 were from HIV negative blood donors from the lake zone blood bank. All HIV positive specimens were screened and documented their results based on Tanzania rapid test algorithm while all HIV negative specimens were proved by 4th generation Enzyme Linked Immunosorbent Assay (ELISA) (Bioelisa HIV1/2 ver.4.0-Spain). The kit has ability to detect antigen (protein 24) IgM and IgG antibodies specific for HIV1/2 viruses with the window period of up to two weeks.

PBS was prepared by using commercially available tablets (Sigma Aldrich Co., USA) whereby one tablet (1814.5-2005.5 mg/tablet) was used in dissolution time of ≤10.0 minutes in 200 ml of deionized water. This produced 0.01M PBS, 0.0027M potassium chloride and 0.13M sodium chloride, pH 7.4 at 25°C. The buffer solution was then autoclaved at 121°C, pressure 1.01 bars for 30 minutes. The buffer was then allowed to cool to room temperature and dispensed in sterile caped universal bottles. The pH of the buffer was monitored before and after autoclaving and every day before analysis of the specimens. The buffer was then stored at room temperature to meet the conditions of resource limited settings. The specimens to be analyzed were free from haemolysis and bench life of 2-10 days before analysis.

Data were recorded in Microsoft excel sheet and Kappa statistics was used to calculate percentage agreement and disagreement between 0.01M PBS and Alere Determine® manufacturer buffer (Landis & Koch, 1977). Ethical approval was granted by Catholic University of Health and Allied Sciences/Bugando Medical Centre Research Ethics and Review Committee (CREC). Permissions were granted by care and treatment centre (CTC) Bugando and Lake Victoria Zone Blood Bank. Of the total specimens tested, 150 (100%) of HIV positive were positive by using both Alere Determine® buffer and 0.01M PBS while 150(100%) of HIV negative samples were negative by both Alere Determine® and 0.01M PBS. Sensitivity and specificity of 0.01M PBS compared to Alere Determine® buffer was 100%. The value of kappa indicates perfect agreement between 0.01M PBS and Alere Determine® buffer (100%)(Table2).

Table1: Distribution of HIV results by Alere Determine® HIV rapid test using Alere buffer and 0.01M PBS

Alere Determine® buffer			
0.01 MPBS	Negative	Positive	Total
Negative	150	0	150
	100%	0%	100%
Positive	0	150	150
	0%	100%	100%
Total	150	150	300
	100%	100%	100%

For the first time this study documents usefulness of 0.01M phosphate buffered saline (PBS) as a detection buffer for HIV Alere Determine® rapid test. PBS has been approved for human and animal therapeutic use as well as in different biological research in clinical settings. It has been useful in maintaining pH in biochemical assays such as immunoassays (WHO, 2005).

Alere Determine® HIV rapid tests are now accepted and widely used as it can be performed in resource limited settings. These tests are usually packed with buffer namely 'chase buffer' of 2.5ml volume. Most of the time this volume is not enough making the good number of the kits not useful or necessitate re-

ordering to cater for the need. In a number of occasions buffers supplied with other kits such as malarial rapid diagnostic test, other rapid tests or distilled water are used instead (Gillet et al., 2010). This has been proved to give a good number of false positive and false negative results. Our findings may provide alternative means of diagnosing HIV with maintained quality of results that can lead to proper management of these patients especially in resource limited settings instead of re-ordering that may increase the running costs.

Based on these findings we recommend the use of 0.01M PBS where the shortage of buffer arises for Alere Determine® HIV rapid test for the whole blood samples. This can be useful in hospital setting and for research purposes where large number of samples is to be analysed. This study confirms that PBS provides the same reliable results as the Alere Determine® buffer supplied by manufacturers. Conversely, rapid detection with 0.01M PBS is simple, inexpensive, not time consuming and sensitive alternative which can be made locally in resource limited settings within 6 hours. In addition, PBS can provide results within a short time of receiving specimens in the same manner as buffers supplied with Alere Determine® tests. Investigation to evaluate the suitable buffer for other rapid tests for different diseases is recommended especially in resource limited settings.

Competing interest

None declared

Author's contributions

SEM, BRK and MMM participated in the design of the work. MFM, LS and CAM participated in the collection of specimens and laboratory work. BRK performed statistical analysis and interpretation of the data. MMM wrote the first draft of the manuscript. All authors read and approved the final version of the manuscript.

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