ASSESSMENT OF THE DIAGNOSTIC PERFORMANCE OF HIV RAPID TEST KITS
IN GHANA

BY

DENNIS ADADE OKYERE
10552314

THIS DISSERTATION IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF
MASTER OF CLINICAL TRIALS DEGREE

JULY, 2017
DECLARATION

This dissertation is the result of my own independent work under the supervision of Dr. Priscillia Awo Nortey with due acknowledgement made to all reference sources. I declare that this work either in whole or in part has not been presented for the award of any degree nor is currently being submitted in candidature elsewhere for another degree.

DENNIS ADADE OKYERE      DATE
(PRINCIPAL INVESTIGATOR)

DR. PRISCILLIA NORTEY      DATE
(SUPERVISOR)
DEDICATION

This dissertation is dedicated to my dear parents, Madam Faustina Awuah and Mr Godfried Adade Okyere for their unflinching support and encouragement. It is dedicated to my siblings as well as my special friends for their support and encouragement.
ACKNOWLEDGEMENT

My sincerest gratitude goes to my academic supervisor, Dr. Priscillia Awo Nortey, for her immense assistance and guidance throughout the work. I am also grateful to the lecturers at the Department of Epidemiology and Disease Control for their contribution towards the successful completion of this work.

My sincere appreciation also goes to Dr. Bismark Sarfo (Head of Department, Epidemiology and Disease Control) and Dr. Francis Anto (Department of Epidemiology and Disease Control) for their assistance towards the successfully completion of this work.

I also appreciate the immense assistance given by Maj LX Adusu-Donkor (37 Military Hospital), BMS Abdul-Karim Haruna (Pentecost Hospital) and BMS Hephzibah Efua Otchere-Baffour (Korle Bu Teaching Hospital) for the successful completion of the work.

I am also grateful to the management and staff of the National AIDS Control Programme and the National Public Health Reference Laboratory for their cooperation and support.
ABSTRACT

Rapid diagnostic test kits are a crucial component of HIV diagnosis in resource limited settings. HIV RDTs are recommended because they are less expensive, require little expertise, require little sample volume and generate results in less than 20 minutes. The WHO, CDC, and UNAIDS recommend countries to undertake pre and post market evaluations of HIV RDTs used for HIV testing in that particular country. Little is known about post market evaluations of test used in Ghana. Most of these test kits are used as single test in HIV prevention and control programmes.

To strengthen HIV screening in Ghana, a laboratory-based cross sectional analytic study was conducted using 200 HIV positive and HIV negative banked samples from the National Public Health Reference Laboratory of The Ghana Health Service at the Korle Bu Teaching Hospital.

The sensitivity of the First Response, Wondfo, Healgen, Advance Quality (Intec) and Fastep test kits were 99.0% each. Advance Quality (Intec) demonstrated a sensitivity of 97%. The specificity of the First Response, Wondfo, Healgen, Advance Quality (Intec) and Fastep test kits were 92.0%, 95.0%, 98.0%, 99.0% and 95.0% respectively. The study results show that the test kits evaluated are good for screening purposes.
LIST OF TABLES

Table 3.1: Test kits evaluated and their manufacturers............................................ 22
Table 4.1: Summary of the performance of the test kits............................................ 33
Table 4.2: Sensitivities and specificities of the test kits............................................ 34
Table 4.3 The positive and negative predictive values of the test kits.......................... 36
LIST OF FIGURES

Figure 1.1: Conceptual framework ................................................................. 4
Figure 2.1: Appearance of HIV immunological markers post infection............. 10
Figure 4.1: The overall performance (accuracy) of the test kits............................ 37
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Content</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td>i</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>DEFINITION OF TERMS</td>
<td>xii</td>
</tr>
<tr>
<td>CHAPTER ONE</td>
<td>1</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Background</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Problem Statement</td>
<td>2</td>
</tr>
<tr>
<td>1.3 Conceptual Framework</td>
<td>4</td>
</tr>
<tr>
<td>1.3.1 Conceptual framework explained</td>
<td>5</td>
</tr>
<tr>
<td>1.4 Justification</td>
<td>6</td>
</tr>
<tr>
<td>1.5 Objectives</td>
<td>7</td>
</tr>
<tr>
<td>1.5.1 General Objective:</td>
<td>7</td>
</tr>
<tr>
<td>1.5.2 Specific Objectives:</td>
<td>7</td>
</tr>
<tr>
<td>CHAPTER TWO</td>
<td>8</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>8</td>
</tr>
<tr>
<td>2.1 The Human Immunodeficiency Virus</td>
<td>8</td>
</tr>
<tr>
<td>2.2 Appearance of virological and Immunological markers post HIV infection</td>
<td>8</td>
</tr>
<tr>
<td>2.3 HIV Testing</td>
<td>10</td>
</tr>
<tr>
<td>2.4 Diagnostic assays for HIV</td>
<td>11</td>
</tr>
<tr>
<td>2.5 Algorithms for HIV testing</td>
<td>12</td>
</tr>
</tbody>
</table>
3.8 Blinding .......................................................................................................................... 26
3.9 Laboratory Reference Assay .......................................................................................... 26
3.10 The INNO-LIA Score Assay .......................................................................................... 26
3.11 Inno-lia Test principle .................................................................................................... 27
3.12 External Quality Control .............................................................................................. 28
3.13 Data Collection Technique ............................................................................................ 28
  3.13.1 Testing Procedure ................................................................................................... 28
  3.13.2 Data collection tool ................................................................................................ 29
  3.13.3 Data Processing and Analysis ................................................................................. 29
  3.13.4 Statistical Methods ................................................................................................. 30
3.14 Pilot Study ...................................................................................................................... 31
CHAPTER FOUR......................................................................................................................... 32
RESULTS ..................................................................................................................................... 32
  4.1 The performance of the Test Kits ................................................................................... 32
  4.2 Sensitivities and Specificities of the test kits ................................................................. 34
  4.3 Positive and Negative Predictive values of the Test kits ............................................... 35
CHAPTER FIVE .......................................................................................................................... 38
DISCUSSION ............................................................................................................................... 38
  5.1 Sensitivities of the test kits............................................................................................. 38
  5.2 Specificities of the test kits............................................................................................ 38
  5.3 Positive and negative predictive values ......................................................................... 39
  5.4 The diagnostic performance of the test kits ................................................................. 39
CHAPTER SIX ............................................................................................................................. 41
CONCLUSION AND RECOMMENDATIONS ......................................................................... 41
  6.1 Conclusion...................................................................................................................... 41
6.2 Recommendations ........................................................................................................ 41
REFERENCE ...................................................................................................................... 43
APPENDIX .......................................................................................................................... 48
LIST OF ABBREVIATIONS

AIDS - Acquired Immuno Deficiency Syndrome
ART - Antiretroviral Therapy
CDC - Center for Disease Control and Prevention
CD4 - Cluster of Differentiation 4
EIA - Enzyme Immuno Assay
ELISA - Enzyme Linked Immuno Sorbent Assay
HCT - HIV Testing and Counselling
HIV - Human Immuno Deficiency Virus
IgG - Immunoglobulin G
IgM - Immunoglobulin M
LCR - Ligase Chain Reaction
NACP - National AIDS Control Programme
NAT - Nucleic Acid Test
NPV - Negative Predictive Value
PCR - Polymerase Chain Reaction
PMTCT - Prevention of Mother- to- Child Transmission
PPV - Positive Predictive Value
RDT - Rapid Diagnostic Test
RNA - Ribonucleic Acid
WB - Western Blot
WHO - World Health Organization
# DEFINITION OF TERMS

**Antibody** - A protein produced by the B-lymphocytes in response to an antigen or infectious agent.

**Antigen** - A substance that induces the production of antibodies or immunologic response.

**Confirmatory test** - A test to confirm or rule out a medical condition in an individual with concerning symptoms or an out-of-range screening result.

**False negative** - A test result that indicates that a person does not have a disease or condition when the person actually does have it.

**False positive** - A test result that indicates that a person does not have a disease or condition when the person actually does have it.

**False positive** - An error in some testing process in which a condition tested for is mistakenly found to have been detected.

**Immunochromatography** - Assay method used in simple devices intended to detect the presence or absence of a target analyte in sample without the need for specialized and costly equipment.

**Negative Predictive Value (NPV)** - The probability that when a test is negative, a specimen does not have antibody to HIV.

**Parallel algorithm** - Testing process in which all specimens are tested initially with two HIV RDT kit simultaneously and discordant results are retested with a third HIV RDT kit.

**Positive Predictive Value** - The probability that when the test is reactive, the specimen
(PPV) actually contains antibody to HIV

Screening test - Laboratory tests that help to identify people with increased risk for a condition or disease before they have symptoms

Specificity - The ability of an assay being evaluated to correctly detect specimens that do not contain antibodies to HIV

Sensitivity - The ability of an assay being evaluated to correctly detect specimens containing antibodies to HIV.

Serial algorithm - Specimens are tested with a single HIV RDT kit and those that test reactive undergo a retesting with a second HIV RDT kit to confirm HIV infection

Seroconversion - The time period during which a specific antibody develops and becomes detectable in the blood

Seropositive - The results of a blood test that show the presence of a specific antibody in the blood serum

Viraemia - A medical condition where viruses are in the bloodstream of an individual

Window period - The time between exposure to HIV infection and the point when the test will give an accurate result
CHAPTER ONE

INTRODUCTION

1.1 Background

The Human Immunodeficiency Virus (HIV) is the causative agent for Acquired Immunodeficiency Syndrome (AIDS). Since 1981 when the first cases of AIDS were reported, the pandemic has claimed the lives of many adults and children throughout the world. The estimated number of people diagnosed and living with HIV in the world is 36.9 million. Out of this number, about 70% (25.8 million) of those infected are in Sub-Saharan Africa. Also, 2.1 million people are newly infected with HIV every year and out of this number, 1.8 million are in Sub-Saharan Africa. The high number of new HIV cases in Sub-Saharan Africa poses a public health concern (UNAIDS, 2015; WHO, 2015).

Timely and accurate diagnosis and management of the HIV infection is crucial to reduce morbidity, mortality and onward transmission to other individuals (Kilembe et al., 2012). The routine use of conventional Enzyme Linked Immuno-sorbent Assay (ELISA) is technically demanding, and requires sophisticated, regularly maintained equipment and constant supply of electricity. This method is also expensive, time consuming and takes a long time for clients to receive their results. There has however been an improvement in HIV diagnosis in the past few decades (Buttò, Suligoi, & Fanales-Belasio, 2010).

Testing for HIV has been made simple and fast now through the use of rapid test kits. In 1992, the World Health Organization (WHO) first recommended the use of rapid detection tests (RDTs) for HIV (Kroidl et al., 2012). Rapid HIV test kits that do not need the use of instruments have been widely used especially in resource limited settings including Africa (Lyamuya et al., 2009). Rapid HIV diagnostic tests as compared to standard ELISA testing are simpler to perform, do not require laboratory facilities, are cost-effective, have a longer shelf life, and allow
point of care provision of HIV testing and counseling (HTC) with increased client linkage to care (Cabie, Bissuel, Huc, Paturel, & Abel, 2011).

A number of rapid HIV antibody test kits have become available in the past ten years. These tests have to a great extent improved access to and ease of HIV testing (Piwowar-Manning et al., 2011). The broad class of HIV RDTs includes lateral-flow (immunochromatographic) and vertical-flow (immunofiltration) assay formats, which detect the presence of HIV-1/2 antibodies and/or HIV p24 antigen (WHO, 2015; Sands, 2015).

With the advancement of technology, HIV RDTs now have very high sensitivities (>98%) and high specificities (>99%) (Lyamuya et al., 2009; Omoding, Katawera, Siedner, & Boum, 2014). Validation of these rapid test kits is in conformance to good clinical practice since there is variation in the subtypes of HIV (Piwowar-Manning et al., 2011).

Even with the advancement of technology and the development of more sensitive HIV RDTs, some infections still remain undetected owing to the fact that laboratory test kits are imperfect (Cohen, Gay, Busch, & Hecht, 2010). Assessment of rapid diagnostic test kits for screening HIV are essential when they are put on the market to ensure that their performances are up to standard. This assessment will help in eliminating or reducing the incidence of misdiagnosis of HIV (Piwowar-Manning et al., 2011, Lyamuya et al., 2009).

This study therefore assessed the post market diagnostic performance of different brands of rapid HIV test kits in Ghana.

1.2 Problem Statement

WHO endorses the use of rapid HIV test kits in resource limited settings for HIV screening (WHO, 2004). This has led to the influx of a number of HIV test kits on the African market
The sensitivity and specificity of the test kits should be taken into consideration before including them in screening algorithms. The World Health Organisation (WHO) has set the specificity and sensitivity of rapid HIV test kits at >98% and >99% respectively (WHO, 2015). Post market evaluation of HIV RDTs is important to ensure quality in the use of these test kits (Branson et al., 2014; Wang et al., 2007).

Evaluation of various anti-HIV assays using panels of American and European sera have shown that most of these assays have a high sensitivity and specificity. Studies in Africa have shown that some of these assays do not have a similar test performance when used for testing of African sera (Banwat, Peter, & Egah, 2009; Lyamuya et al., 2009; Moal et al., 2014).

A number of rapid HIV test kits have been imported onto the Ghanaian market and are used by both private and public health care facilities. The National AIDS Control Programme (NACP) recommends the use of the First Response test kit for screening clients for HIV. There has been no published post market assessment of the HIV RDTs test kits that are commercially available on the Ghanaian market after extensive literature search.

Post market evaluations will ensure quality assurance of HIV testing. This may reduce the chance of false negative and false positive cases and save time and money in running expensive confirmatory tests. Ultimately, the safety of blood in our various Blood Banks and the general Public Health may be improved. It is fulfilling to explore ways of improving HIV RDTs to make them perform better and helping to attain the goals of HIV prevention and control programmes in Ghana.

For the reliability of the results given out, this study seeks to evaluate the post market diagnostic performance of some brands of the rapid HIV test kits available in Ghana.
1.3 Conceptual Framework

Figure 1.1: The Conceptual Framework for diagnostic performance

- **Brand of Test Kit**
- **Integrity of Test Kit**
- **Storage of test kit**
- **Competency of Laboratory Scientist**
- **Type of Sample**
  1. Serum
  2. Plasma
  3. Whole blood
  4. Oral fluid
- **Test Outcome**
  1. Reactive
  2. Non reactive
- **The Stage of Infection**
  1. Early stage
  2. Window period
  3. AIDS stage
1.3.1 Conceptual framework explained

The quality of results given out to patients when rapid test kits are used depends greatly on the brand of test kit used, the integrity of the test kit, the type of sample used and the competence of the person conducting the test.

The brand of test kits may affect the outcome of the test in that there is a standard that all brands of test kits should meet to be able to perform very well. But the brands could either be brands that are standard or sub standard. This can affect the quality of results produced by the test kit. The integrity of the test kit can also affect its performance. These test kits are all imported into the country and the nature in which they are transported could affect it. Also, the storage of the test kits in the warehouses could affect their integrity which in the long run may affect test results from these test kit (Sands, 2015). The type of the sample being analysed could also affect the test results. Samples such as whole blood, serum, plasma, oral fluid or urine can be used (Galiwango et al., 2013). The competency level of the laboratory scientist performing the test can also affect the outcome of the testing process. Depending on the stage of the infection, the test kit may or may not be able to identify the antibodies to the Human Immunodeficiency Virus (HIV). Different stages are characterized by different immunological markers which can be detected by different assay methods (Buttò et al., 2010).
1.4 Justification

The use of complex and expensive high sensitive HIV testing is rarely sustainable in sub-Saharan Africa due to economic and logistic constraints. The use of HIV RDTs is therefore inevitable even though there are documentations showing some levels of limitations (Boeras et al., 2011).

A study reports that in sub-Saharan Africa, 3% of people undergoing HIV testing at out-patient departments are at risk of receiving false negative results (Bassett et al., 2011). Non detection of these individuals could be attributed to failure of test kits. Studies in some other parts of the world also established the tendency of HIV RDTs to give false positive results (Banwat et al., 2009; Ndase et al., 2015). Failure of test kits arises from different factors including inappropriate ambient temperature and storage of the kits and the brand of test kits (Wesolowski et al., 2006; Amechi, Osagie, & Chikwendu, 2006).

These are some of the reasons why The Centre for Disease Control and Prevention and WHO advice countries to do post market assessments of HIV RDTs that are used within the country (Aphl, 2004). Post evaluations on HIV RDTs have been carried out in most countries (Wang et al., 2007; Wesolowski et al., 2006; Cabie et al., 2011).

In Ghana, NACP recommends First Response HIV test kit to be used for screening clients in health centres. There are other HIV RDTs commercially available in Ghana. With the recent power outages, storage of these kits may also be affected. This may affect the integrity of the rapid HIV test kits hence affecting the quality of results obtained from using them.

In view of all these factors and the need for post market assessment of test kits, the study seeks to assess the post market diagnostic performance of the test kits. This will ultimately help in decisions that will improve Public Health.
1.5 OBJECTIVES

1.5.1 General Objective:
To evaluate the diagnostic performance of HIV rapid test kits on the Ghanaian market.

1.5.2 Specific Objectives:
The specific objectives of the study are:

1. To determine the specificity of each test kit
2. To determine the sensitivity of each test kit
3. To compare the diagnostic performance of the test kits to ELISA, which is the clinical standard HIV testing procedure in Ghana.
CHAPTER TWO

LITERATURE REVIEW

2.1 The Human Immunodeficiency Virus

The human immunodeficiency virus (HIV) is a lentivirus and belongs to the family Retroviridae. HIV is an enveloped virus and has two positive sense RNA strands as its genetic material (Santos and Soares, 2010). Retroviruses are characterized by the possession of the enzyme reverse transcriptase, which allows viral RNA to be transcribed into DNA and incorporated into the host cell genome by the help of another enzyme, integrase. Glycoproteins on the surface of HIV bind to the target cell and help the virus to enter the cell. The virus encodes multiple regulatory proteins, which control the life cycle and viral expression (Frankel and Young, 1998).

2.2 Appearance of virological and Immunological markers post HIV infection

When an individual is exposed to the virus, the virus begins to attack the host’s CD4 cell and begin replication. At this stage of the infection, the only viral marker present in the host’s blood is the viral RNA (viraemia), which is not detectable by most commercially available rapid test kits because the RDTs determine the presence of antibodies. Antibodies are not present at this stage of HIV infection. The viral p24 antigens also appear which can be measured in blood after only 11-13 days of the infection. The p24 antigen concentration continues to remain high in the serum of the individuals six weeks after infection (Buttò et al., 2010). The period where only the viral RNA and viral p24 antigens are present is termed the serological window period. During this period, there are high levels of viral RNA and viral p24 antigen but no detectable HIV-
specific antibodies in the blood stream of infected individuals. The window period ends when HIV–specific antibodies are detected in the individual’s bloodstream (Buttò et al., 2010).

Human immune responses to viral antigens begin on the third week of infection and continue through to the 24th week (Tomaras et al., 2008). The individual becomes seropositive when antibodies to the Human Immunodeficiency virus becomes detectable in the blood. This marks the end of the window period. In most cases, the first HIV-specific antibodies to appear are IgM, usually within the first three weeks from infection and peak between the 3rd and the 4th week. However, response is strongly dependent on each individual’s immune system (Chaix et al., 2013). HIV-specific IgG antibodies usually appear at about 4-5 weeks after infection. The concentration of viral antigens reduces as the amount of antibodies specific to the virus begins to increase. The virus replicates very faster at the final stage which is the AIDS stage (Bonarek et al., 2006)
Figure 2.1 shows the various HIV markers and how they appear and peak at different stages of the infection.

Fig 2.1: Appearance of HIV immunological markers post infection

2.3 HIV Testing

HIV testing is a process of establishing the HIV status of an individual. The process tests for antibodies specific to HIV or the viral antigens in the individual’s blood. HIV testing and counseling is now recognized as a priority in national HIV programmes because it forms the gateway to HIV/AIDS prevention, care, and treatment and support interventions (Bassett et al., 2011). The process of HIV testing involves the identification of markers associated with the
virus. These markers can be immunological (specific antibodies to HIV) or virological (viral antigen or viral RNA) that become present in the body fluids of the person. Whole blood, saliva and blood components such as serum or plasma can be used for testing for the Human Immunodeficiency Virus (Buttò et al., 2010)

2.4 Diagnostic assays for HIV

There are two methods of HIV diagnosis namely: Qualitative and Quantitative. Qualitative methods are used to check for the presence or absence of the HIV antibodies. There are two categories under qualitative methods. These are screening assays and confirmatory assays. Individuals who have the disease are identified among the population using screening tests. Those who test reactive for the screening tests are confirmed with a testing method known as confirmatory assays. As a consequence, screening tests must have a high degree of sensitivity (low false negative rate), whereas confirmatory assays must possess a higher specificity (low false positive rate). Most screening and confirmatory tests must be performed sequentially so that the results that are generated can be said to be reliable and accurate (Fanales et al., 2010). The quantitative method of HIV diagnosis is used to estimate the number of viral antigen present in the blood of the individual (Berger, Preiser & Doerr, 2001).
2.5 Algorithms for HIV testing

To increase reliability and accuracy of HIV test results, a set of testing algorithms have been developed which enables the two assay types to be performed in a number of combinations (Gurtler, 1996).

Strategies in HIV testing where more than one rapid diagnostic test kits are used in the detection of an HIV infection is known as HIV testing algorithm. There are two algorithms that are widely used. They are the serial and parallel HIV testing algorithms. In the serial algorithm, all specimens are tested with a single HIV RDT kit and those that test reactive undergo a retesting with a second HIV RDT kit to confirm HIV infection. Specimen that test non-reactive on the first HIV RDT kit but test reactive on the second HIV RDT kit are considered indeterminate. The second is the parallel algorithm in which all specimens are tested initially with two HIV RDT kit simultaneously and discordant results are retested with a third HIV RDT kit. The serial and parallel test algorithms are used for HIV diagnosis and in HIV prevalence studies (WHO, 1992). Ghana has adopted the serial testing algorithm for HIV screenings in the country.

2.6 Rapid Diagnostic HIV testing (HIV RDTs)

Rapid diagnostic test kits are simple, instrument-free tests that are used for measuring antibodies by enzyme-linked immunoassay (EIA). A number of rapid HIV tests are now available. These test kits are also referred to as rapid or simple test devices. The principles underlying these test are one of the following. These are particle agglutination, immunodot (dipstick), immunofiltration and immunochromatography. The most predominantly used principle is the immunochromatography (Buttò et al., 2010; Ribeiro-rodrigues et al., 2003).
In order to avoid venipuncture and centrifugation of samples, whole blood from capillary blood is used. This can be obtained from the finger tip. The test results are normally available within 20-30 minutes. Other sample types such as oral fluid, serum, plasma or urine can also be used in HIV RDT. Immunologic techniques basically follow the same pattern in the test kits. There is a portion of the test strip coated with antigens to HIV-1 and HIV-2 and a procedural control portion that checks correctness of test done (Branson et al., 2014) Daskalakis 2011. A positive result is indicated by a coloured dot or band at the test band or an agglutination pattern. The test results are normally visually read and are suitable for use in testing and counseling centres and in resource limited settings. RDTs normally test for the presence of antibodies to HIV but recent advancements allow both antibodies and antigens to be screened at a goal. This dual technology is known as the fourth generation rapid test kits (Buttò et al., 2010; Crucitti, Taylor, Beelaert, Fransen, & Damme, 2011).

2.7 Enzyme Immunoassays (EIA) / Enzyme-linked Immuno-sorbent assay (ELISA)

The principle on which most of the antibody assays depend is the specific antigen-antibody reaction.

Enzyme-linked Immunosorbent Assays (ELISAs) for the identification of antibodies to HIV were first introduced in 1985 (van der Groen, Van Kerckhoven, Vercauteren, & Piot, 1991).

The EIA has undergone several modifications with the aim of improving sensitivity and lowering the window period of HIV diagnosis (Chappell et al, 2009).
Fourth generation HIV RDTs are now commercially available for use. The first, second and third generations of EIAs detect HIV-specific antibodies whiles the fourth generation detects both HIV-specific antibody and viral antigen (Murphy & Aitken 2011). The first and second generations are designed with whole viral lysates and synthetic polypeptides respectively to detect the antibody-IgG in a patient’s serum produced against HIV. The antibody (IgG) reacts with the antigen (whole viral lysate or synthetic polypeptide) to form an antigen-antibody complex in a micropipette plate. The complex serves as a substrate in a reaction with a second antibody that contains an enzyme molecule. The substrate-enzyme complex produces a colour (optical density) which registers on a spectro-photometer. The optical density values are then used to calculate test cut-offs to separate positive values from negative ones. This technique is capable to detect infection 35-40 days after viral exposure. This generation has modifications in the test principle, an enzyme is linked to the HIV antigen (conjugate) to which an HIV-specific antibody in the patient’s serum will bind. A substrate is then introduced to facilitate a reaction with the enzyme in the conjugate. The enzyme-substrate reaction produces a colour change in a plate well containing the mixture. This is the ELISA principle, which the third and fourth generations are built on (Brust et al., 2000). The assay detects both IgG and IgM HIV-specific antibodies and has a relatively higher sensitivity and specificity. The window period after which HIV infection can be detected are reduced to 22 days.

The more recent fourth generations are able to detect IgG, IgM and viral p24 antigens. With this enhanced design, HIV infection is detected within 12 days after viral infection. Fourth generation ELISAs have higher sensitivity and specificity and are comparable to assays that estimate the quantities of viral RNA (Buttò et al., 2010)
2.8 p24 antigen assays

The HIV viral core is made up of proteins known as the p24 antigen. The amount of p24 antigen are in an individual’s serum is high in the first few weeks of infection (Weber, 2006). One characteristic HIV antigen is a viral protein termed as the p24. This is a structural protein that forms most of the HIV viral core (Bentsen et al., 2011).

Antibodies to p24 are produced during the conversion of antigens to antibodies. P24 antigens are therefore not detectable after seroconversion. Therefore, p24 antigen assays are not reliable for diagnosing HIV infection after its early stages. However, HIV infection can be accurately diagnosed earlier with combined antibody/antigen tests than with purely antibody-detecting tests (Brennan et al. 2013). p24 antigens are now incorporated in most fourth generation HIV testing assays to increase the early and accurate detection of early infections with HIV. This has proven to be a breakthrough in the fight against HIV/AIDS epidemic (Bentsen et al., 2011; Ramos, Harb, Dragavon, & Coombs, 2013; Schüpbach, 2002; Weber, 2006)

2.9 Factors affecting the performance of HIV rapid diagnostic test kits

Most rapid diagnostic test kits rely on the production of visible colour changes produced by antibody-antigen reactions within the test region of the test kit and read by eye. Documented studies have pointed out certain factors that influence the test results of HIV RDT kits (Ndase et al., 2015). HIV RDT kits are known to form faint bands irrespective of the sample type used and are at times difficult to interpret. A study showed that in some situations weak bands can be so faint to an extent that they are reported as negative test results (Lyamuya et al., 2009)
There is a study that portrays the notion that in regions where there is a high ambient temperature, various infective agents are predominant in the blood stream of the population resulting in high false positive HIV test results (Gould & Higgs, 2009). Some other antibodies produced in certain disease conditions may cross-react with the antigens coated onto HIV RDT kits. Cross-reactivity is common in pregnant women, autoimmune patients, renal failure disease, recent influenza vaccinations and people with other viral infections. Usually cross-reactivity causes false positive RDT results (Mahajan et al., 2012). Some have suggested that HIV RDT performance is influenced by the geographic location of the test setting. It is reported by that HIV RDT kits tend to turn out more false positive results in lower altitudes than in higher altitudes (Lyamuya et al., 2009).

A very important aspect of quality assurance in HIV testing is the performance of pre and post market evaluations on the test kits (Aphl, 2004; WHO, 2004). Pre and post market assessments are part of measures to curb misdiagnosis of HIV. Post market evaluation of test kits have been done in some countries and seen to improve the quality of HIV testing (Wang et al., 2007; Wesolowski et al., 2006).

2.10 Failure of rapid kits

In the quest to improve diagnostic performance and also minimize false negatives, it is recommended by the World Health Organization (WHO) that countries undertake pre-evaluation of rapid kits before they use them in their settings. The WHO also recommends that post market evaluations should be conducted to maintain a high standard diagnostic tool in HIV testing. However, this is not properly practiced in Africa since there seem to be limited published documents indicating an extensive validation of HIV rapid kits against Gold standard tests.
Initially, it was believed that the inaccuracy of HIV rapid tests was attributed to test-settings but this assertion has been refuted by studies across Africa. Results from studies in some parts of Africa have showed high rates of false negatives among children who were on Anti-Retroviral Therapy (ART). After initially testing negative on HIV rapid kit, about 5% of pregnant women were later confirmed to be HIV positive (Bassett et al., 2012).

2.11 Confirmatory assays for HIV

2.11.1 Western blot

Indeterminate HIV screening results, confirmatory tests are performed on samples to settle any doubts. One of such confirmatory assays is the Western blot (WB). Western blot is often used in investigations to isolate and identify proteins. In this method a combination of proteins is separated based on molecular weight, and thus by type, through gel electrophoresis. These results are then transferred to a membrane producing a band for each protein. The membrane produced is then incubated with labels antibodies specific to the protein of interest. Any antibody that is unbound is then washed off leaving only the antibody that has been bound to the protein of interest. The bound antibodies are then detected by developing the film. A colorimetric reaction will show the presence of HIV proteins recognized by antibodies. This is shown as “bands” on the strip. The quantity of protein present corresponds to the width of the bands; thus doing a standard can indicate the amount of protein present (Banwat et al., 2009; Buttò et al., 2010; Scientific, n.d.).
The western blot technique was initially thought of as the best assay (tie-breaker) to confirm HIV infection in indeterminate samples from screening tests but WB assay possess a challenge of subjectivity in results interpretation, it produces much indeterminate results compared to other confirmatory tests (Tebourski, Slim, & Elgaied, 2004).

Detection of viral materials in an HIV exposed individual.

2.11.2 Polymerase Chain Reaction (PCR)/ Nucleic Acid technology (NAT) and Viral Load

The human immune mechanisms take a long time to produce antibodies against the HIV. The delay in seroconversion posed a threat to the fight against HIV. Technologies have been advanced to aid in the detection of HIV infection as early as 6 days after an individual is exposed to the HI virus. PCR was developed by Karry Mullis as a research tool but has been incorporated into clinical diagnosis due to its effectiveness (Nicole Ngo-Giang-Huong Woottichai Khamduang Baptiste Leurent1, 2, 2011; Weber, 2006).

PCR technology is used to amplify a single copy or a few copies of a piece of the viral RNA across several orders of magnitude, generating thousands to millions of copies. RNA circulating freely outside blood cells can be detected by different laboratory technologies. Nucleic acid tests (NAT) are based on Polymerase Chain Reaction (PCR), branched DNA (b-DNA), Nucleic Acid Sequence-Based Amplification (NASBA), ligase Chain Reaction (LCR), or real-time PCR techniques. In situations where acute infection is suspected and antibodies are undetectable by antibody tests especially in newborns of infected mothers, the NAT test is used as a supplementary test to diagnose HIV infection; such as in early infant diagnosis for infants born to HIV mothers who have gone through preventing mother-to-child transmission (PMTCT) HIV programme. PCR and nucleic acid technology have inadvertently increased the early detection of
HIV (Branson et al., 2014; Buttò et al., 2010; Weber, 2006; Daskalakis 2011). The use of PCR can detect exposure to HIV as early as 6-7 days of infection, because newer PCR technologies are able to detect as little as 20 copies of HIV in an individual’s blood sample. The number of viral copies in a person’s blood is termed as viral load (Pas et al., 2010; Stephan, Hill, Hadacek, van Delft, & Moecklinghoff, 2014).

2.12 CD4 estimation

A cluster of differentiation 4 (CD4) count is a laboratory test that measures the number of CD4 T lymphocytes (CD4 cells) in a blood sample. In people with HIV, it is the most important laboratory indicator of how well your immune system is working and the strongest predictor of HIV progression. CD4 cells (often called T-cells or T-helper cells) are a type of white blood cells that play a major role in protecting the body from infection. CD4 cells send signals to activate the body’s immune response when they detect antigens like the HIV (Bonarek et al., 2006; Kiragga et al., 2014).

Once a person is infected with HIV, the virus begins to attack and destroy the CD4 cells of the person’s immune system. HIV uses the machinery of the CD4 cells to multiply (make copies of itself) and spread throughout the body. The CD4 count of an uninfected person who is generally in good health ranges from 500 cells/µl to 1,200 cells/µl. A very low CD4 count (less than 200 cells/µl) is one of the ways to determine whether a person living with HIV has progressed to stage 3 infection (AIDS) (Bhattar et al., 2014; Kiragga et al., 2014). Therefore, WHO (2013) recommends antiretroviral therapy (ART) initiation at CD4 threshold of ≤ 500cells/µl. Patients with CD4 cell count below or above this threshold remain infectious and carry detectable viral RNA in their plasma (Donnell et al., 2010).
In HIV diagnosis, it is believed that rapid test kits can detect HIV-specific antibodies in the blood of patients on prolonged ART and have achieved CD4 counts greater than 350 cells/µl. (Stevens et al, 2010).
CHAPTER THREE

METHODS

3.1 Study Design

This study was post market surveillance. The study was a cross-sectional study in which a selected number of samples stored at the Nation Public Health Reference Laboratory were used as panel of samples to evaluate HIV rapid test kits on the Ghanaian market.

A laboratory based qualitative experiment was performed on the test kits to be assessed.

3.2 Rapid HIV test kits evaluated and their manufacturers

The National AIDS Control Programme (NACP) requires that First Response as the test kit to be used for screening and the OraQuick rapid HIV test kit as supplementary to the First Response. There are other test kits that were available on the Ghanaian market for use by the public. A search of the medical supply stores in the country revealed 18 HIV other rapid test kits that were on the market.

The First Response four (4) test kits were selected from the pool of eighteen test kits that were collated from the market. In selecting the four test kits, all the 18 test kits were numbered, and by balloting four numbers were selected. The test kits that corresponded to the selected numbers were then used in the study. In all, five (5) test kits were evaluated. The First Response (Premier Medical Corporation Ltd., Kachigam, India) test kit was provided by the National AIDS Control Programme. The rest of the test kits were purchased from the suppliers on the market. The instructions in the package inserts of each of the rapid test kit were followed for the testing procedure. Table 3.1 shows test kits evaluated and their manufacturers.
Table 3.1: Test kits evaluated and their manufacturers

<table>
<thead>
<tr>
<th>Name of Test kit</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Response</td>
<td>Premier Medical Corporation Ltd, India</td>
</tr>
<tr>
<td>Wondfo</td>
<td>Guangzhou Biotech Co. Ltd, China</td>
</tr>
<tr>
<td>Healgen</td>
<td>Zhejiang Orient Gene Biotech Co, China</td>
</tr>
<tr>
<td>Advance Quality (Intec)</td>
<td>Intec Products Inc, China</td>
</tr>
<tr>
<td>Fastep</td>
<td>Polymed Therapeutics Inc, USA</td>
</tr>
</tbody>
</table>

3.3 Variables

3.3.1 Dependent (Outcome Variables)

Outcome of the test:

I. **Negative test result** occurs when only the control band of the test kit shows on the test kit 15 minutes after sample and reagent application.

II. **Positive test result**, occurs when either HIV-1 or HIV-2 or both HIV-1 and HIV-2 bands and the control band shows on the test kit 15 minutes after sample and reagent application.

III. **Invalid test result**, occurs when only the test band or neither test band nor the control bands of the test kit shows after 15 minutes of sample and reagent application.

3.3.2 Independent Variables

i. Brand of test kit

ii. Serum samples

iii. Competency of laboratory scientist
iv. Integrity of test kit
v. Stage of infection
vi. Storage of test kits

3.4 Sample Population

The study was a laboratory based study using banked HIV positive and HIV negative sera. These are sera stored at the Public Health Reference Laboratory which is located on the premises of Korle Bu Teaching Hospital. The samples are HIV sentinel survey HIV positive and HIV negative samples which have been stored at the Public Health Reference Laboratory. Therefore, these are samples from all over the country.

3.5 Ethical Issues

Ethical approval was sought from the Ghana Health Service Ethical Review Board (Ethical certificate number: GHS-ERC: 67/12/15).

An agreement of confidentiality was signed between the authorities of the National AIDS Control Programme (NACP) and the Principal Investigator. The data collected from these samples were kept confidential and only the PI had access to them. Permission was also sought from the Head of the Public Health Reference Laboratory at Korle Bu Teaching Hospital before beginning the study. The study posed no risk to any individuals but has a greater benefit to the NACP and its programmes.

All investigations that were conducted on the samples will contribute to the patient diagnosis and management.
3.6 Sampling

3.6.1 Sample Size Calculation

The sample size was calculated using the sensitivity (98%) and specificity (99%) values quoted in the World Health Organization’s criteria for selection of HIV rapid test kits and the national HIV prevalence (1.3%) to estimate the positive predictive and negative predictive values. The positive predictive value is 96% and the negative predictive value is 99%. This means there is a 4% probable positive case that will not be detected by the kit. The predictive values were computed in a sample size calculator (Epi Info version 7) to determine the sample size in which this difference could be detected at a power of 80% and a significance level of 0.05. The sample size is 200 samples after correcting for possible spillages and insufficient samples.

3.6.2 Panel of Samples

The National Public Health Reference Laboratory conducts sentinel surveys across Ghana, therefore they have stored samples which are invariably a representation of the population of the country. Therefore the samples that were used for this assessment could be said to be nationwide in nature. These samples are stored at -80°C and will remain in their original good state for fifteen years. Specimens were randomly selected to include 100 HIV -positive and 100 HIV negative samples from the Public Health Reference Laboratory at the Korle Bu Teaching Hospital.

3.6.3 Selection of the panel of samples used for the evaluation

To evaluate the rapid test kits, samples or sera from the National Public Health Reference Laboratory (NPHRL) were used. The National AIDS Control Programme (NACP) in
conjunction with the NPHRL tests Ghanaians for HIV in their sentinel surveys. The sera used for the study were obtained from the archived samples at the NPHRL which is situated within the premises of the Korle-Bu Teaching Hospital. These are samples taken from selected local clinics around the country. These samples give a good representation of the country geographically.

There were over two thousand HIV positive samples archived from the 2016 HIV survey. All these samples had pathological numbers on them. These numbers were entered into Epi Info Version 7. A command was given for the computer to generate one hundred numbers from the pool of numbers that were entered. Samples that corresponded to these numbers were selected and used as the true positives in the study.

The same selection procedure was used in the selection of the one hundred HIV negative samples from the pool of archived samples. These samples served as the true negative samples in the study. All the banked samples at the National Public Health Reference Laboratory (NPHRL) have been tested using the INNO-LIA assay method. This is the gold standard being used in the clinical setting in Ghana. Two hundred samples were used in the assessment of the test kits.

Each test kit was analyzed on the entire two hundred samples; therefore, there were 200 HIV results for each test kit.

### 3.6.4 Samples Storage

All samples selected to be used for the experiment were stored at -70 °C to keep their integrity of the samples until they were ready to be used. Samples will be thawed to room temperature before testing is done.
3.7 Pre testing refresher training
Before the main testing is undertaken, the two Biomedical Scientists (BMS) who took part in the testing of the test kits were taken through refresher training at the PHRL. The BMS were refreshed on the standard operating procedures (SOPs) on rapid HIV testing. They were also taken through safety and quality control measures.

3.8 Blinding
Investigators (Biomedical Scientists; myself and another Biomedical Scientist from Korle Bu Teaching Hospital) blinding was used in this study. This means that the reference results of the test was not made available to the laboratory scientist performing the analysis on the rapid test kits. The results were only provided by the reference laboratory to the Principal Investigator after analysis was done and results computed by the laboratory scientist. This was done to help minimize or remove any form of bias by the person performing the testing.

3.9 Laboratory Reference Assay
The Gold standard for HIV testing in Ghana’s clinical settings is the Enzyme Linked Immunosorbent Assay (ELISA). The INNO-LIA HIV I/II Score Assay method was employed at the Public Health Reference Laboratory as the reference assay method. This is the method used by the NPHRL for HIV I/II confirmation.

3.10 The INNO-LIA Score Assay
The INNO-LIA™ HIV I/II Score is a Line Immuno Assay used to confirm the presence of antibodies against the Human Immunodeficiency Virus type 1 (HIV-1), including group O, and type 2 (HIV-2) in human serum or plasma. The INNO-LIA™ HIV I/II Score also differentiates
between HIV-1 and HIV-2 infections. It is intended as a supplementary assay on specimens found to be reactive using an anti-HIV screening procedure.

3.11 Inno-lia Test principle

Recombinant proteins and synthetic peptides from HIV-1 and HIV-2, and a synthetic peptide from HIV-1 group O are coated as discrete lines on a nylon strip with plastic backing. Five HIV-1 antigens are applied: sgp120 and gp41, which detect specific antibodies to HIV-1, and p31, p24, and p17, which may also cross-react with antibodies to HIV-2. HIV-1 group O peptides are present in the HIV-1 sgp120 band. The antigens gp36 and sgp105 are applied to detect antibodies to HIV-2. In addition to these HIV antigens, four control lines are coated on each strip: anti-streptavidine line, ± cut-off line (human IgG), 1+ positive control line (human IgG) and one strong 3+ positive control line which is also the sample addition control line (anti-human Ig). The INNO-LIA™ HIV I/II Score is based on the enzyme immunoassay principle (EIA). The test sample is incubated in a test trough together with the multiple antigen-coated test strip. HIV antibodies, if present in the sample, will bind to the individual HIV antigen lines on the strip. Afterwards, a goat antihuman IgG labeled with alkaline phosphatase is added and will bind to any HIV antigen/antibody complex previously formed. Incubation with enzyme substrate (BCIP/NBT) produces a dark brown color in proportion to the amount of HIV antibody present in the sample. Color development is stopped with sulfuric acid. If the sample contains no HIV-specific antibodies, the labeled antihuman antibody will not be bound to antigen/antibody complex so that only a low standard background color develops.
3.12 External Quality Control

For monitoring the quality of frozen storage, a percentage of specimens were retested with a nucleic acid test (PCR) prior to performing the evaluation to ensure that the integrity of the sample are still intact post storage.

Twenty (20) samples were randomly selected and tested by a third Biomedical Scientist at Korle Bu Teaching Hospital Central Laboratory to serve as quality check on the testing results.

The data collected were entered on a computer and also on external storage devices (external hard drives, pen drives and compact discs) as backups.

3.13 Data Collection Technique

3.13.1 Testing Procedure

Specimens which have already been tested for presence or absence of HIV and have been stored at the Public Health Reference Laboratory (PHRL) at the Korle Bu Teaching Hospital were used for this experiment. All the samples that were selected for the evaluation were tested on each of the five selected test kit.

The testing was done by qualified laboratory scientists. The Biomedical Scientists were one qualified Biomedical Scientist from the PHRL and me.

Before testing, all the samples to be worked on a particular day were thawed at room temperature.

Specimens were tested on each test kit according to the manufacturer’s instructions by one laboratory scientist blinded to the reference results.
The HIV RDT test results were interpreted and recorded on a laboratory data record sheet. The second blinded study laboratory scientist independently interpreted and recorded the test result within 1 minute of the first reading. Discrepancies were resolved with a third Biomedical Scientist. Reasons for discrepancies were duly noted. Invalid tests were repeated using a new test and recorded as such.

The testing was performed in a well-lit laboratory at the National Public Health Reference Laboratory. The results of the RDTs were recorded as positive, negative or invalid; and positive band strength was noted. Weak-positive bands are defined as samples with a positive band that is lighter than the positive control band on the test strip. A positive result was defined as both the sample and the control line of the test developing; and a negative result was defined as only the control band developing. Invalid results were defined as no band developing and/or only the development of a band in the sample test area and none in the control zone.

3.13.2 Data collection tool
All results from the analysis were entered onto a laboratory result sheet (log book) developed purposely for the project. After all the testing was done, all the data was transferred onto Microsoft Excel Spreadsheet. This was then extracted onto StataSE (version 13.1) for analysis.

3.13.3 Data Processing and Analysis
StataSE (version 13.1, StataCorp, Texas, USA) is the statistical software that was used to analyse the data.
Categorical variables were coded numerically in StataSE (version 13.1, StataCorp) for easy analysis.

### 3.13.4 Statistical Methods

All categorical variables were analysed as proportions/percentages with 95% confidence intervals.

The sensitivity of each test kit with the sera was determined. A Chi-square test was performed to determine the significance of difference between the various test kits.

The sensitivity was calculated as the proportion of infected samples that were picked by the test kit as positive expressed as a percentage. The numerator was the number of HIV infected samples that tested positive with the test kit and the denominator was the total number of HIV infected samples.

The specificity of the test kits were calculated as the proportion of un-infected samples that were correctly identified by the test kit as negative expressed as a percentage. The numerator was the number of HIV un-infected samples that the test kit identified as negative and the denominator was the total number of HIV un-infected samples.

The Positive Predictive Value (PPV) was calculated and expressed as a percentage. The numerator was the number of true positives (sensitivity x prevalence of disease) and the denominator was the number of test positives (true positives + false positives).

The Negative Predictive Value (NPV) was also calculated and expressed as a percentage. The numerator was the number of true negatives (specificity x 1-prevalence of disease) and the denominator was the number of test negatives (true negatives + false negatives).
3.14 Pilot Study

A pilot study was conducted at the Central Laboratory of Korle Bu Teaching Hospital to assess the procedures that were used for the proposed study. The study methodology and data collection tools were assessed to determine how best they capture relevant variables for analysis. Corrections were made to any procedural portions that had any shortfall. The results were assessed with the statistical methods intended for the study to determine shortcomings in study methodology and quality control procedures.
CHAPTER FOUR

RESULTS

HIV rapid test kits offer a good means by which early diagnosis can be done. In order to achieve accurate and dependable results, test kits used in this regard should be evaluated at the sites where they are being used. This study therefore sought to evaluate the diagnostic performance of five (5) selected test kits that are being used in Ghana. In doing so, the sensitivities, specificities, the positive and negative predictive values and the accuracy of each of the test kits was assessed. The work was carried out at the National Public Health Reference Laboratory (NPHRL) in Ghana in May 2017.

4.1 The performance of the Test Kits

The results of the various test kits after being subjected to the two hundred (200) samples is summarized in Table 4.3 on page 33.

There were 100 samples that were clinically positive and confirmed with the gold standard (INNO-LIA). These samples are what is termed as the true positive samples. The First Respose, Wondfo, Healgen and Fatep correctly identified 99 out of the 100 positive samples. Advance Quality however was able to identify 97 out of the 100 positive samples. The rest of the true positives that were identified by the test trips as negative is what we term as false negative, hence: the First Respose, Wondfo, Healgen and Fatep produced 1 false negative each. The Advance Quality produced 3 false positives.

There were also 100 samples that were clinically negative and confirmed with the gold standard (INNO-LIA). These samples are what is termed as the true negative samples. The First Response, correctly identified 92 out of the 100 negative samples. The Wondfo correctly identified 95 out of the 100 negative samples. The Healgen correctly identified 98 out of the 100
negative samples. Advance Quality was able to identify 99 out of the 100 positive samples. Fastep correctly identified 95 out of the 100 negative samples. The rest of the true negatives that were wrongly identified by the test strips as positive is what we term as false positive, hence: the First Response produced 8 false positives, Wondfo produced 5 false positives, Healgen produced 2 false positives, Advance Quality produced 1 false positive and Fastep also produced 5 false positives.

Table 4.1: Results of the test kit

<table>
<thead>
<tr>
<th>Test kit</th>
<th>GOLD STANDARD (INNO-LIA)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>Total</td>
</tr>
<tr>
<td>First Response</td>
<td>Negative</td>
<td>92</td>
<td>1</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>8</td>
<td>99</td>
<td>107</td>
</tr>
<tr>
<td>Wondfo</td>
<td>Negative</td>
<td>95</td>
<td>1</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>5</td>
<td>99</td>
<td>104</td>
</tr>
<tr>
<td>Healgen</td>
<td>Negative</td>
<td>98</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>2</td>
<td>99</td>
<td>101</td>
</tr>
<tr>
<td>Advance Quality (Intec)</td>
<td>Negative</td>
<td>99</td>
<td>3</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>1</td>
<td>97</td>
<td>98</td>
</tr>
<tr>
<td>Fastep</td>
<td>Negative</td>
<td>95</td>
<td>1</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>5</td>
<td>99</td>
<td>104</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100</td>
<td>100</td>
<td>200</td>
</tr>
</tbody>
</table>
4.2 Sensitivities and Specificities of the test kits

The World Health Organization (WHO) sets 98% as the minimum sensitivity for a test kit to be said to be diagnostically effective in screening of patients.

The sensitivity of a test kit is the ability of the test kit to correctly detect specimens containing antibodies to the Human Immunodeficiency Virus (HIV). At the end of the study, four of the test kits: First Response, Wondfo, Healgen and Fastep demonstrated 99.0% sensitivity each. The Advance Quality however demonstrated a sensitivity of 97%. Overall, the sensitivities were marginally below the stated values stated in the package inserts (Table 4.2).

<table>
<thead>
<tr>
<th>Test Kit</th>
<th>Sensitivity (%)</th>
<th>95% CI</th>
<th>Specificity (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Response</td>
<td>99.0</td>
<td>94.6 – 99.9</td>
<td>92.0</td>
<td>84.8 – 96.5</td>
</tr>
<tr>
<td></td>
<td>Package insert</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Wondfo</td>
<td>99.0</td>
<td>94.6 – 99.9</td>
<td>95.0</td>
<td>88.7 – 98.4</td>
</tr>
<tr>
<td></td>
<td>Package insert</td>
<td>99.1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Healgen</td>
<td>99.0</td>
<td>94.6 – 100</td>
<td>98.0</td>
<td>93.0 – 99.8</td>
</tr>
<tr>
<td></td>
<td>Package insert</td>
<td>99.5</td>
<td>99.8</td>
<td></td>
</tr>
<tr>
<td>Advance Quality</td>
<td>97.0</td>
<td>91.5 – 99.4</td>
<td>99.0</td>
<td>94.6 – 100</td>
</tr>
<tr>
<td></td>
<td>Package insert</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Fastep</td>
<td>99.0</td>
<td>94.6 – 99.9</td>
<td>95.0</td>
<td>88.7 – 98.4</td>
</tr>
<tr>
<td></td>
<td>Package insert</td>
<td>99.9</td>
<td>99.4 – 100</td>
<td>&gt;99.8</td>
</tr>
</tbody>
</table>

*CI = Confidence interval

The specificities of a test kit is the ability of the test kit to correctly detect specimens that do not contain antibodies to HIV. The World Health Organization (WHO) quotes the specificity a good
HIV rapid test kit to be at a minimum of 99%. The specificities of the test kits is shown in Table above.

### 4.3 Positive and Negative Predictive values of the Test kits

Table 4.5 shows the Positive Predictive Values (PPV) and Negative Predictive Values (NPV) of the five test kits that were assessed. The Negative Predictive Value of a test kit is defined as the probability that when a test is negative, the specimen does not have antibody to HIV. The higher the NPV, the more true negatives the test kit is likely to produce and vice versa. The test kits assessed showed various degrees of Negative Predictive values; First Response, Wondfo, Healgen, Advance Quality (Intec) and Fastep were 98.9%, 98.9%, 99.0%, 97.0% and 99.0% respectively.

The Positive Predictive Value of a test kit is the probability that when the test is reactive or positive, then the specimen actually contains antibody to HIV. The five test kits demonstrated varying positive predictive values. The lower the PPV, the higher the number of false positives that the test kit is likely to produce and vice versa. First Response, Wondfo, Healgen, Advance Quality and Fastep demonstrated 92.5%, 95.2%, 98.0%, 99.0% and 95.2% positive predictive values.
Table 4.3: Positive and negative predictive values of the test kits

<table>
<thead>
<tr>
<th>Test Kit</th>
<th>Positive Predictive value</th>
<th>95% CI (%)</th>
<th>Negative Predictive value</th>
<th>95% CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Response</td>
<td>92.5%</td>
<td>86.4 – 96.0</td>
<td>98.9%</td>
<td>92.9 – 99.9</td>
</tr>
<tr>
<td>Wondfo</td>
<td>95.2%</td>
<td>89.4 – 97.9</td>
<td>99.0%</td>
<td>93.1 – 99.9</td>
</tr>
<tr>
<td>Healgen</td>
<td>98.0%</td>
<td>92.6 – 99.5</td>
<td>99.0%</td>
<td>93.3 – 99.9</td>
</tr>
<tr>
<td>Advance Quality</td>
<td>99.0%</td>
<td>93.2 – 99.9</td>
<td>97.1%</td>
<td>91.5 – 99.0</td>
</tr>
<tr>
<td>Fastep</td>
<td>95.2%</td>
<td>89.4 – 97.9</td>
<td>99.0%</td>
<td>93.1 – 99.9</td>
</tr>
</tbody>
</table>

*CI = Confidence interval

4.4 Overall performance (Accuracy) of the test kits

Accuracy of a test kit is a measure of how close a test result is to the standard or the true value. In other words, it shows the overall performance of each test kit. The purpose of using rapid test kits is to screen the population to be able to find out the individuals who are infected with a particular disease so that they could be managed well. Therefore, the tool used for this purpose should be able to give a result that is a representation of the standard or demonstrate the true nature of the sample: whether diseased or not. Healgen and Advance Quality demonstrated high accuracy of 98.5% (95% CI: 96.8-100) and 98.0% (95% CI: 96.6-99.9). Wondfo and Fastep showed an accuracy level of 97.0% (95% CI: 94.6-99.4) each. The First Response test kit demonstrated the lowest accuracy among the five test kits evaluated with a 95.5% accuracy level. Figure 4.6 shows the overall diagnostic performance of the various test kits.
Figure 4.1: Shows the overall performance (accuracy) of each test kit.
CHAPTER FIVE

DISCUSSION

The five (5) HIV rapid test kits assessed had high sensitivities but the values were below the values that were stated in the package inserts for the test kits. Even though some brands had package insert of 100% sensitivity and 100% specificity, none of the test kit exhibited 100% sensitivity and 100% specificity. All the test kits performed well in the study.

5.1 Sensitivities of the test kits

Four of the test kits; First Response, Wondfo, Healgen and Fastep demonstrated sensitivities of 99.0% (95% CI: 94.6-99.9) each which was greater than 98% which is the sensitivity that a good test kit should at least have according to the World health organization (WHO HIV RDT Guidelines, 2015). This means that the four test kits have the ability to correctly detect antibodies to the HIV if they are present in an individual’s blood. In Advance Quality however had 97% (95% CI: 91.5-99.4) sensitivity which is a little below the WHO set target.

5.2 Specificities of the test kits

The test kits exhibited varying specificities. This demonstrates that the ability of the test kits to correctly detect the absence of antibodies to the Human Immunodeficiency Virus in serum sample that are tested with them. Advance Quality and Healgen had specificities of 99.0% (95% CI: 94.6-100) and 98.0% (95% CI: 93.0-99.8) respectively. Both Wondfo and Fastep had specificities of 95% (95% CI: 88.7-98.4). First response had specificity of 92% (95% CI: 84.8-96.5) which happened to be the test kit with the least sensitivity among the five(5) test kits.
evaluated. Therefore Healgen and Advance Quality can be said to be able to correctly identify more negative sera than the other.

There was no sample that gave a false-positive or false-negative result on more than one test kit, so that, had two (2) kits been used to test samples in parallel. A result which emphasize the importance of using two (2) different HIV rapid antibody test kit to reduce the events of false-positive or false-negative results for patients.

5.3 **Positive and negative predictive values**

All test kits evaluated had high Negative Predictive Values which gives assurance that the kits have higher probabilities to indicate if sample is clinically negative. Wondfo and Fastep had low Positive Predictive Value, 95.2% (95% CI: 89.4-97.9) each. However, First Response demonstrated the lowest Positive Predictive Value of 92.5% (95% CI: 86.4-96.0%). First response is therefore likely to give more false-positive results as than the rest of the test kits making users bear more cost on the user to run more confirmatory tests.

5.4 **The diagnostic performance of the test kits**

The test kits evaluated demonstrated high accuracy. Healgen demonstrated the highest accuracy of 98.5%, followed by Advance Quality: 98.0%. Wondfo and Fastep demonstrated 97.0% accuracy each. First Response had the lowest accuracy level with a value of 95.5%. Therefore, the five test kits assessed are not the only HIV rapid test kit on the Ghanaian market readily available for use and on the results of this study may not apply to the other test kits. Again, the HIV subtypes of the samples were not determined. Therefore, it is not known whether the
sensitivities and specificities would have been different if different mix of HIV subtypes were used. Again the differences in the calculated sensitivities and specificities of the test kits and the values quoted in the package inserts may be due to the difference in panels of sera used in the evaluation.

A higher number of the test kits evaluated showed good accuracy, short turnaround time and needed minimal infrastructure and human resource, which is good for testing HIV in this part of the world.
CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This evaluation of the diagnostic performance of 5 test kits showed that the Healgen HIV rapid test kit demonstrated the highest diagnostic performance and First Response the poorest diagnostic performance. The First Response is the HIV rapid test kit that is used widely as the screening tool for the National AIDS Control Programme (NACP).

All the test kits evaluated had good sensitivities which mean that they have the capacity to correctly identify individuals with HIV if tested with the kits. The Advance Quality test kit demonstrated the highest specificity and First Response demonstrated the lowest specificity. The specificity, the most important attribute of a screening tool, is the ability of the test kit to correctly detect individuals who are negative.

6.2 Recommendations

To The National AIDS Control Programme (NACP)

1. There should be regular post marketing assessment of all the test kits available on the Ghanaian market and any HIV test kit that subsequently imported into the country.

2. There should be a reassessment of the diagnostic performance of the First Response since it demonstrated the lowest specificity and low Positive Predictive Value.
To the Food and Drugs Authority

3. There should be proper records keeping of all HIV test kits that have been approved to be sold on the Ghanaian market.

4. The authority should also conduct random post market assessments on the test kits.
REFERENCE


Buttò, S., Suligoi, B., & Fanales-belasio, E. (2010). Laboratory diagnostics for HIV infection, (Figure 1), 24–33. http://doi.org/10.4415/ANN


APPENDIX

NATIONAL AIDS/STI CONTROL PROGRAMME (NACP)

P. O. Box KB 547
Korle-Bu, Accra
Tel. (233-302) 67 84 57 - 9
Fax: (233-302) 66 26 91
Email: info@nacp.org.gh
April 27, 2017

RE: SUPPORT OF DATA SHARING AGREEMENT:
MR. DENNIS ADADE OKYERE AND NACP

We wish to state that the National AIDS/STI Control Programme (NACP) has signed a data sharing contract with Mr. Dennis Adade Okyere, an MSc Clinical Trials student of the School of Public Health, to enable him acquire data for his research activities. The research is on “Post Market Assessment of the Diagnostic Performance of HIV Test Kits in Ghana”.

In line with the above, please be informed that the NACP has agreed to allow Mr. Dennis Adade Okyere the right of limited access to the datasets for the research activities using Archived HIV Samples from the National Public Health and Reference Laboratory.

He may therefore go ahead with the said research taking into consideration the conditions in the agreement.

We count on your cooperation.

DR. STEPHEN AYISI ADDO
PROGRAMME MANAGER

THE HEAD
NATIONAL PUBLIC HEALTH & REFERENCE LAB.
KORLE – BU
ACCRA

cc: Mr. Dennis Adade Okyere
Department of Epidemiology and Disease Control
School of Public Health
University of Ghana
APPENDIX

THE NATIONAL AIDS/STI CONTROL PROGRAMME (NACP)
DATA SHARING AGREEMENT WITH COLLABORATOR

AN AGREEMENT FOR ACCESS
TO HIV/AIDS/STI DATA ARCHIVED BY NACP

PREAMBLE

With the accumulation of data by the National AIDS/STI Control Programme, many requests are being received from researchers to access datasets produced by the National AIDS/STI Control Programme. It has therefore become important for the National AIDS/STI Control Programme to develop a controlled and efficient system to manage access to these datasets. This agreement hereby sets out to achieve that purpose.

This agreement is undertaken by the undersigned research collaborator of the National AIDS/STI Control Programme (NACP) AND the NACP and it is aimed at providing access to specific data for the purpose of collaborative research between the two parties and shall come into force on the date of execution of this agreement as indicated hereunder.

The parties hereby agree as follows:

1. That the National AIDS/STI Control Programme (NACP) remains the legal custodian of the said data.
2. That the following documents must accompany this agreement:
   a. A short description of the intended purpose and method of analysis of the data (an analysis plan).
   b. A list of names and organizational affiliations of all those who will engage in this analysis. In the case of a student a statement by his/her supervisor that they will ensure that the student abides by these conditions.
   c. A description of the means by which the investigator will restrict access to confidential NACP data.
   d. A signed confidentiality agreement form, annexed to this agreement.

That NACP has agreed to allow the undersigned collaborator right of limited access to the data sets for a research on the topic “Post Market Assessment of the Diagnostic Performance of HIV Rapid Test Kits in Ghana”

3. That the undersigned collaborator has agreed not to disseminate the said data or any sub-sample thereof to any third party, except with the written permission of the Programme Manager of NACP. The undersigned research collaborator assumes direct responsibility for any unauthorized or illegal access to the data entrusted to her/him/it or its agents.
4. That any investigator acting for or on behalf of the undersigned research collaborator, shall not release or permit others to release any data that would in any way disclose the identity of persons captured in the data, directly or indirectly.
5. That the undersigned collaborator has agreed to send to the Programme Manager, for review before publication and for the agreement of the two parties, a draft of each paper or any publication to be produced as part of the research.
6. That the undersigned collaborator has agreed to acknowledge the use of the NACP database on each paper or publication produced as part of the research, and such acknowledgement must first be approved by the Programme Manager of NACP.

7. That the undersigned collaborator has agreed to transfer to the Programme Manager of NACP and delete all the copies of the data after completing the research.

8. That the undersigned collaborator has agreed to deliver all copies of the data to the Programme Manager of NACP.

9. That the data remain the property of the National AIDS/STI Control Programme which shall reserve the right to request the return of the dataset to NACP upon breach of any of the above stated terms and conditions by the undersigned research collaborator.

10. That any dispute arising out of this agreement shall be amicably settled by the two parties; and where there is no agreement as to the settlement of the matter, the matter shall be referred to an arbitrator agreeable to both parties, for the settlement of the dispute.

The two parties, NACP and the undersigned research collaborator, hereby sign this agreement to affirm their strict adherence to the terms and conditions herein.

1. SIGNED: ........................................
   PROGRAMME MANAGER
   NATIONAL AIDS/STI CONTROL PROGRAMME
   P. O. BOX KB 547
   KORLE-BU, ACCRA
   Date: 09/05/17

2. SIGNED: ........................................
   MR. DENNIS ADIADOKYERE
   SCHOOL OF PUBLIC HEALTH,
   UNIVERSITY OF GHANA
   Date: 09/MAY/2017
NATIONAL AIDS/STI CONTROL PROGRAMME (NACP)

In case of reply the number and date of this letter should be quoted
My Ref. nacp/gen./vol.15
Your Ref. No..........................................

P. O. Box KB 547
Korle-Bu, Accra
Tel. (233-302) 67 84 57 - 9
Fax: (233-302) 66 26 91
Email 1: naddo@nacp.org.gh
Email 2: info@nacp.org.gh

April 27, 2017

NACP CONFIDENTIALITY UNDERTAKING

AN ANNEX TO THE NATIONAL AIDS/STI CONTROL PROGRAMME (NACP)
DATA SHARING AGREEMENT WITH COLLABORATOR (AN AGREEMENT FOR
ACCESS TO HIV/AIDS/STI DATA ARCHIVED BY NACP)

THIS IS AN UNDERTAKEN MADE BY MR. DENNIS ADADE OKYERE, AN
MSC. CLINICAL TRIAL STUDENT IN THE DEPARTMENT OF
EPIDEMIOLOGY AND DISEASE CONTROL OF THE SCHOOL OF PUBLIC
HEALTH - UNIVERSITY OF GHANA, TO ENSURE CONFIDENTIALITY UNDER
THE AFORE-MENTIONED AGREEMENT FOR A RESEARCH ON THE TOPIC
“POST MARKET ASSESSMENT OF THE DIAGNOSTIC PERFORMANCE OF
HIV RAPID TEST KITS IN GHANA”

The purpose of this Undertaking is to ensure that NACP can secure the confidentiality of any
information it provides under this agreement. It is required that trust is built to allow for current
and future program of joint work collaboration, and collective publication to proceed effectively
in good faith. This Undertaking must be signed by all persons, including reviewers of manuscripts
for publication, who shall be given access to any data of NACP in the course of the duties under
this agreement.

1. In line with the terms and conditions of the agreement:
   i. the NACP, may grant the undersigned access to the information in its
      custody in the course of his/her participation in above mentioned
      research/project activities.
   ii. the NACP is willing to provide to the undersigned the information for the
      explicit purpose of performing his/her responsibilities under this agreement,
      provided only that the undersigned undertakes to disclose the information
      only to persons who are bound by like obligations of confidentiality and non-
      disclosure as contained in this undertaking.

2. The Undersigned undertakes to regard the information as confidential and
   proprietary to the NACP and agrees to take all reasonable measures to ensure
that the Information is not used, disclosed, or copied, in whole or in part, other
than as provided in paragraph 1 above, except that the Undersigned shall not be
bound by any such obligation if he/she is clearly able to demonstrate that the
Information:
i. was known to him/her prior to any disclosure by NACP to the Undersigned; or
ii. was in the public domain at the time of disclosure by NACP; or
iii. becomes part of the public domain through no fault of the Undersigned.

Signature ___________________________ Name ___________ Date 08/05/17

One signed original for the NACP, one signed original for the Member Site