QATAR UNIVERSITY

COLLEGE OF HEALTH SCIENCES

PERFORMANCE EVALUATION FOR THE HIV AND SYPHILIS DETECTION PROTOCOLS USED BY THE MEDICAL COMMISSION DIAGNOSTIC LABORATORY IN QATAR

BY

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ABSTRACT

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Title: PERFORMANCE EVALUATION FOR THE HIV AND SYPHILIS DETECTION PROTOCOLS USED BY THE MEDICAL COMMISSION DIAGNOSTIC LABORATORY IN QATAR

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Background: The Medical Commission is responsible for screening all newcomers and expatriates in Doha, Qatar to ensure that infectious diseases do not enter the country. The infectious disease tests include; HIV, Hepatitis B, Hepatitis C, Syphilis, and Tuberculosis. Aim: In this study, we aim to evaluate the diagnostic efficiency of the protocols used in the Medical Commission for the diagnosis of HIV and Syphilis using specific statistical measures. Methodology: The replicates of ELISA reading was analyzed and used for repeatability and reproducibility purpose. For RPR testing, a total of 198 syphilis reactive samples during the period of January 2019 to December 2019 were included in this study. Results: HIV screening retrospective data has been collected from a total of 585,587 individuals who visited the Medical Commission, in which 595 were positive for HIV. The assays obtained from these individuals for HIV were analyzed and compared to each other to test the reproducibility and cost-effectiveness of this diagnosis protocol. Two rounds of HIV diagnostic results showed a 99.83% agreement (overall and positive percent agreements; 95% CI, 99.05-99.97%). For syphilis analysis, data were obtained from the RPR test performed on 198 blood samples. In line with this, the overall percent agreement between the two RPR dilution2

and TPA Elisa was 36.55% (95% CI; 30.14%-43.47%), whereas the positive percent agreement was 100% (94.93%-100%). In order to ensure true positives, PPV results were obtained and were found similar to the overall percent agreement of the RPR dilution 2 and TBA ELISA i.e. 36.55% (30.14%-43.47%). Conclusion: The medical commission used highly performance techniques that are reliable and efficient for screening of STDs. Nevertheless, further studies are needed to test the agreement of these tests with other confirmatory tests done at Hamad Medical Corporation.

DEDICATION

I will dedicate this study to my daughters who inspired me to be strong in front of many obstacles in my life

ACKNOWLEDGMENTS

I would like to express my appreciation to my family, for the greatest support, advice, and encouragement. Second, I would like to be grateful to my Supervisor and my committee members for their help and effective comments.

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TABLE OF ABBREVIATIONS

Abbreviation	Meaning
AIDS	Acquired Immunodeficiency Syndrome
STI	Sexually Transmitted Infection
VIF	Viral Infectivity Protein
VPR	Viral Protein R
TAT	Trans activator of Translation
REV	Controller of Viral Protein Expression
NEF	Negative Administrative Figure
VPU	Viral Protein U
VPX	Viral Protein X
MC	Medical Commission
HMC	Hamad Medical Cooperation
HIV	Immunodeficiency Virus
RPR	Rapid Plasma Reagin
PCR	Polymerase Chain Reaction
NAAT	Nucleic Acid Amplification Tests
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid

CHAPTER 1: INTRODUCTION

Sexually transmitted diseases are defined as diseases that are transmitted through sexual contacts. The most common sexually transmitted infections are: Chlamydia, Gonorrhea, Trichomoniasis, Syphilis, and Acquired immunodeficiency syndrome (AIDS) caused by Human Immune Deficiency virus (HIV), Chlamydia trachomatis, Neisseria gonorrhea, Trichomonas vaginalis, and Treponema pallidum. These sexually transmitted diseases cause seriously urogenital symptoms such as cervicitis, urethritis, vaginitis, and genital ulceration, and few of these agents infect the rectum and pharynx (1). HIV and Syphilis are of different prevalence around the world. There are approximately 75 million individuals that have become infected with HIV since the start of the epidemic (2). On the other hand, the worldwide predominance of syphilis was assessed to be 1.11%. Although the percentage of syphilis is considerably low, the predominance of the diseases increases among districts in local areas in Africa (3).

Indeed, each year, there are about 6 million new cases of syphilis globally due to this reason. In recent years, with the presence of HIV infection and its concurrence with syphilis, there has been a great focus on understanding the rates of syphilis and HIV coinfection (4). Such coinfection has been found in several cases, for example nearly 10% of Danish men analyzed with syphilis also acquired HIV infection (5). In addition, HIV-positive patients were found to be associated with sexual behaviors related to syphilis than HIV-negative patients (6). Because of this coinfection incidences, several methods to improve the diagnosis of HIV and syphilis had been implemented.

HIV can be diagnosed by detecting HIV antibodies (7) and/or detecting viral RNA, DNA, enzymes, and proteins (8) or by applying different novel methods (9). On the other hand, Syphilis can be diagnosed using Dark field microscopy, which is the most specific technique for syphilis diagnosis when an active chancre or condyloma latum is present (10). However, its accuracy is limited by the experience of the operator performing the test, the number of live treponemes in the lesion, and the presence of non-pathologic treponemes in oral or anal lesions (11). Thus, the main method for diagnosis of syphilis is non treponemal tests such as the VDRL test and rapid plasma reagin test. However, with these tests, false positive results are common because of pregnancy, autoimmune disorders, and infections (12). Qualitative nontreponemal tests are widely used for syphilis screening. However, their usefulness is limited by decreased sensitivity in early primary syphilis and during late syphilis, when up to one third of untreated patients may be nonreactive (11). Molecular methods such as PCR technology (13) and other novel methods (14) are also used.

According to the Qatar Ministry of Public Health (MOPH), communicable diseases account for around 8% of all deaths in Qatar, negatively affecting the life quality of residents and creating an overwhelming concern for Qatar's healthcare system (15) In addition, with the influx to the county of the high numbers of multi-national expatriates, Qatar continues to face a considerable challenge in infection control. The Medical Commission (MC) in Qatar works under the umbrella of the Ministry of Public Health, which contribute effectively to the implementation of the ministry's general strategy. The MC is responsible for screening newcomers and expatriates to Qatar in order to make sure that infectious diseases, including HIV and Syphilis, do not

enter the country. The Medical authorities in Qatar ensures that the country remains a disease-free nation, requiring individuals by law to take a medical exam upon arrival in the State. The medical exam is essential to protect the health of the entire population as a whole, but this also protects the newcomers themselves and making sure to avoid any spread of infectious diseases.

In this study, we aim to evaluate the performance of the protocols for the diagnosis of HIV and Syphilis in the MC Laboratory. Our work will provide a useful benchmark information to improve the testing protocol in the medical commission to achieve the most reliable results with minimal cost. In addition, our study is the first of its kind to compare the performance of Architect instruments, INNOLIA and Architect, and finally between PCR and Architect diagnostic results for HIV and Syphilis. The aim of this study is to evaluate the performance of the protocols for the diagnosis of HIV and Syphilis in the Medical Commission Laboratory.

CHAPTER 2: OBJECTIVES AND SIGNIFICANCE

The study objective is to analyze and quantify the percentages of the variation between the results of the screening and confirmatory assays. Therefore, we hypothesize that if we could identify the amount and the source of the variation between assays by measuring Replicability and PPV, this will enable us to improve the testing protocol in the medical commission to achieve the most reliable results with minimal cost.

This study is considered significant since it is expected to improve the replicability or reproducibility (in other words, precision) and the positive predictive value (PPV) which is related to sensitivity (since it will detect the probability of a person with positive results and having the disease)) in the Medical commission laboratory and should enhance Laboratory Quality Management System. Besides, this study considered the first of its kind to compare between the performance between Architect instruments, INNOLIA and Architect, and finally between PCR and Architect results as no study evaluated the performance of the abovementioned testing previously.

CHAPTER 3: LITERATURE REVIEW

Sexually transmitted diseases (STD) have been described since the ancient times. The invention of penicillin is a turning point in the history of sexually transmitted diseases and their treatment. The post-penicillin era saw a noteworthy decrease within the rates of sexually transmitted diseases, particularly, syphilis. Likewise, issues of other sexually transmitted diseases of bacterial, fungal and viral etiology came to be tackled well with the invention of generations of highly potent antibiotics, antifungal, and antiviral drugs. Each year, an estimated 500 million people contract one of the four STDs i.e. Chlamydia, Gonorrhea, Syphilis and Trichomoniasis (16). In addition, the burden of these diseases globally increased by the emergence of AIDS.

2.1 Types of Sexually Transmitted Diseases

Sexually transmitted infections are a broad term which includes infections by bacteria, virus, protozoa, fungi, and ectoparasites that result in clinical manifestations involving genitalia and other parts of the body in sexual interaction (16). Of these infective organisms, HIV virus causing AIDS and Treponema pallidum causing Syphilis are considered the most important.

2.2 HIV

2.2.1 Epidemiology of HIV

2.2.1.1 Worldwide

Around 37.9 million individuals are as of now living with HIV where the global predominance among grown-ups (the percentage of individuals ages 15-49 who are infected) has increased since 2001 to reach 0.8% in 2018. Of the individuals infected with HIV in 2018, 36.2 million were adults and 1.7 million were children under age 15 (2). Figure (1) shows the worldwide prevalence of HIV in the year 2018. HIV prevalence is increasing in nearly every geographic region in the world, mostly because of deaths averted from antiretroviral treatment. Although the expansion of antiretroviral

drugs has dramatically improved survival among people living with HIV, yet the prevalence of HIV is high worldwide (17). Figure1 shows the prevalence of HIV worldwide (18)

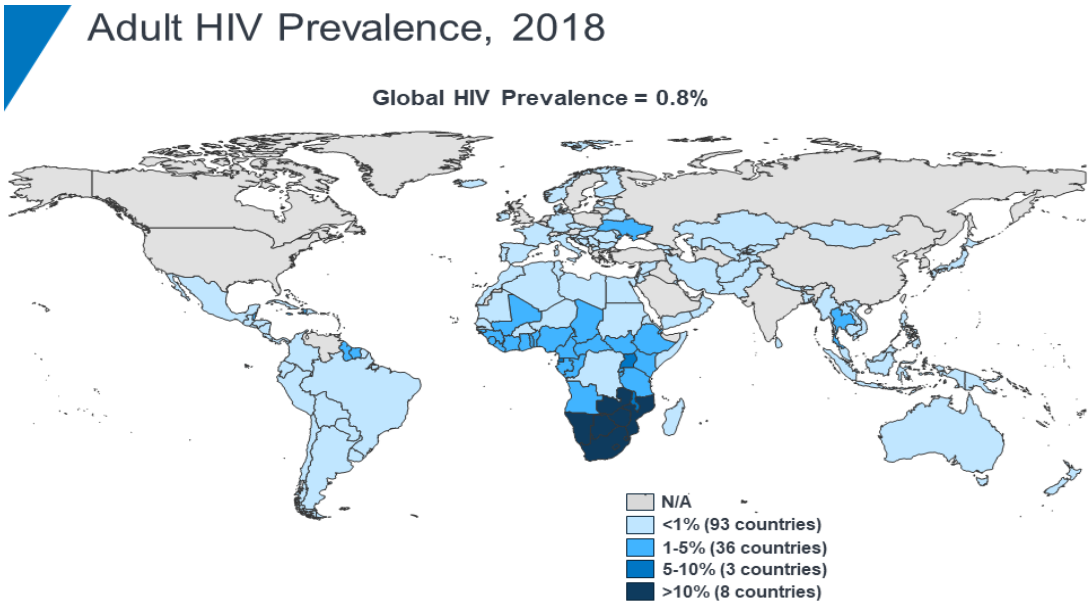

Figure 1: Worldwide prevalence of HIV.

2.2.1.2 Regional

About 5.9 million individuals are living with HIV in Asia and the Pacific, whereas about 240,000 individuals are diagnosed with HIV within the Center, East, and North Africa (2).

2.2.1.3 Qatar

Qatar remains a low prevalence country for HIV infection. The disease affects mainly young male adults with many of them presenting late in the disease. The epidemiology of HIV infection in Qatar has changed over time with infection being mostly sexually transmitted in later years. Only 306 cases of HIV infections in Qatar during the period between 1984 and 2014, indicating that Qatar is a low predominance nation for

HIV infection. The foremost common mode of transmission was sexual i.e. 72% and 43% were due to blood transfusion (19). In addition, the study showed that half of the infected individuals were Qataris and the disease affects mainly young male adults with many of them presenting late in the disease. Importantly, the epidemiology of HIV infection in Qatar has changed over time with infection being mostly sexually transmitted in later years of life (19).

2.2.2 Classification and Structure of HIV

HIV is an enveloped virus of approximately 100 nm in diameter harboring a positive sense single stranded RNA genome. The virus is composed of 2 copies of the ~10 kilobase (kb) positive sense RNA genome in the viral core, the protease, integrase, and reverse transcriptase enzymes (20). The trimeric transmembrane glycoprotein gp41 is embedded in a lipid envelope, to which the surface glycoprotein gp120 is joined. In addition, gp41 and gp120 are two other glycoproteins that help in virus attachment. Beneath that layer, there are; protein p17, the center proteins p24, p6, and the nucleocapsid protein p7 Figure 2 (21). All those proteins are encoded by the viral gag gene. Other proteins include VIF (viral infectivity protein), VPR (Viral Protein R), TAT (Trans activator of Translation), REV (Controller of Viral Protein Expression) and NEF (Negative Administrative Figure). An extra protein found in HIV-1 but not HIV-2 is VPU (Viral Protein U). Additionally, VPX (Viral Protein X) is found in HIV-2 and not HIV-1 (20).

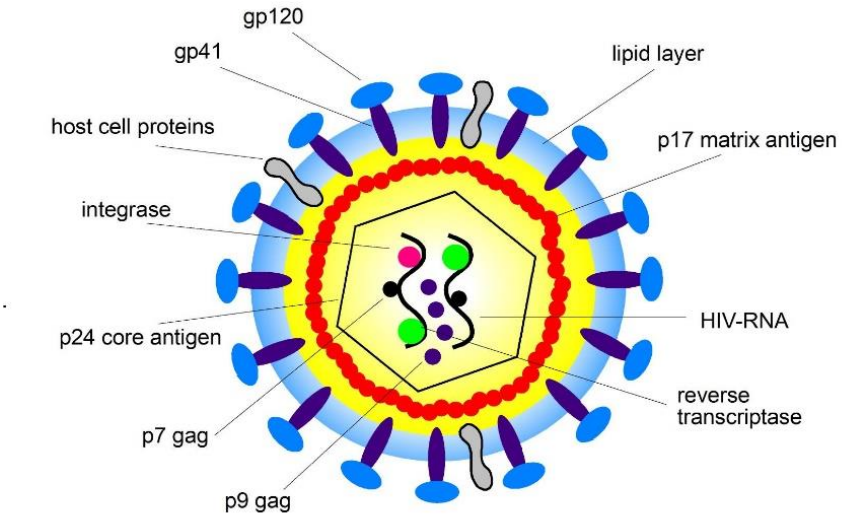

Figure 2:Structure of HIV

2.2.3 Pathogenesis of HIV infection

Till now, HIV infection pathogenesis remains a fascinating topic that requires further studies. Infection with HIV starts without any symptoms or clinical signs, however, it is usually accompanied by slight decrease in the immune system (22). This stage spans up to 3 months after the viral infection until seroconversion where HIV-specific antibodies can be detected in individuals following recent exposure. The outcome of infection and duration for disease progression with clinical symptoms may vary greatly between individuals, but often it progresses fairly slowly (22). It takes several years from primary infection to the development of symptoms of advanced HIV diseases and immunosuppression (22, 23).

During primary infection, although individuals may look healthy, the virus is actively replicating in the patient's lymph nodes and blood stream. Consequently, the immune system will get weaken slowly by the burst of viral load in their bodies (23). Eventually, at later stages, the patient will become susceptible to other opportunistic infections including; Mycobacterium avium, Mycobacterium tuberculosis, Pneumocystis carinii, CMV, toxoplasmosis and candidiasis.

2.2.4 Diagnostic assays for HIV

2.2.4.1 Rapid Tests

These are based on same principle as EIA but provide faster results due to utilization of high antigen concentration and more sensitive color detection reagents.

2.2.4.2 Confirmatory Assays

IFA (Immunofluorescence Assay) has been largely replaced by Western Blotting (WB) and LIA (Line Immunoassays). But unlike EIA, none of these can detect IgM Antibodies or viral antigens and are more expensive. Hence, serial or parallel EIA testing algorithms can suffice at a much-reduced cost in resource-poor settings. The rapid test that comes about is affirmed by Western Blot measure. Although fast tests are fast and simple as they can use capillary blood, plasma, and serum as a sample or even urine or oral secretion, they are less sensitive than other tests. The base of rapid tests is immune-chromatographic methods as well as particle agglutination and immune filtration techniques (24). Besides, usually the HIV positive samples are confirmed by INNOLIA confirmatory test.

The advanced quality HIV Rapid Test is used to diagnose HIV in South Africa to screen for a positive HIV diagnosis. In Bhowan et al study, they tested the performance of the fourth generation of the rapid test and found that it had no better results than the third generation of the same rapid test (7). If a pink line appears in either the HIV antibody window or the HIV p24Ag window or both of the windows, then HIV is positively diagnosed. The test is considered substantial as if the positive control is recognized (7).

2.2.4.3 Architect

One of the automated assays that are based on the EIA idea and is widely used in many countries in the world including Qatar is Architect (Figure 3) (25). ARCHITECT® HIV Ag/Ab Combo is an in vitro chemiluminescent microparticle immunoassay that detects HIV p24 antigen and antibodies to HIV-1 and/or HIV-2 in human serum and plasma. It is fully automated and takes ~30-minutes time to detect the first result. Its overall specificity is 99.77% (95% CI: 99.62-99.88%), HIV antibody sensitivity is 100% (95% CI: 99.63- 100.00%), and HIV p24 Ag explanatory affectability is 18.39 pg/mL (run 17.80-19.68 pg/mL). HIV Combo recognizes HIV diseases during the early, late, and intense stages of the infection (26). The evaluated performance of the ARCHITECT HIV Ag/Ab kit (Abbott) in African setting-Cameroon was found to have 100% sensitivity and a specificity of 97.6% (25).

There are several Architect immunoassays I Abbott types:

1-The ARCHITECT i2000SR immunoassay analyser

- It has a maximum throughput of up to 200 tests per hour
- It features a load-up capacity of 135 samples with 35 priority and 100 routine areas
- It consists of 25 refrigerated reagent positions

2-The ARCHITECT i4000SR immunoassay analyzer

- It offers a maximum throughput of up to 400 tests per hour
- It Features a load-up capacity of 285 samples with 35 priority and 250 routine areas
- It consists of 50 refrigerated reagent positions

Figure 3: Screening by ARCHITECT immunoassay

2.2.4.3 Molecular methods

Molecular Assays are important in HIV diagnosis, and detection of acute infection (9). PCR and NAAT are used for detecting viral RNA, DNA, enzymes, and proteins. These methods are useful for early diagnosis in infants born to seropositive mothers, diagnosing acutely infected individuals in specific periods and assessing viral load for follow-up of infected patients. Using qualitative PCR, viral nucleic corrosive is amplified to distinguish HIV while quantitative RNA, PCR is used to screen HIV-Positive Patients and to choose when HIV treatment ought to begin (24).

Bhowan et al.(2012), stated several advantages for these rapid tests for HIV detection, including ease of administration, ease for patients, rapid results to reduce loss to follow-up and help initiate treatment immediately as well as needing no special instrumentation (7).

They also stated that it costs less than serological tests. These findings have confirmed the superiority of NAAT tests was found over rapid serological tests. The sensitivity for early HIV detection was only 22% -33% compared to a sensitivity of 76%-88% for the fourth generation of NAAT tests. They suggested that programs that use rapid tests for HIV detection switch to NAAT or other antigen-based tests (27).

Also, a one-step TaqMan NAT qRT-PCR strategy for the location of HIV RNA was created where they observed linearity over a range of 1 × 102 to 1 × 107 IU/ml and a lower limit of detection of 100 IU/ml (28). The Cobas Taqscreen MPX test may be a nucleic corrosive enhancement method. This test first isolates the purified nucleic acids from human plasma, and then the second step is the amplification and discovery of HIV-1, HIV-2, HCV RNA, and HBV DNA. Finally, it is actuated by the expansion of manganese acetic acid derivation, turn around translation (for RNA targets) continues, taken after by PCR intensification of exceedingly preserved districts of HIV-1, HIV-2,

HCV RNA, and HBV DNA using specific primers (28). Although the sensitivity of molecular RNA assays is remarkable in acute HIV, their specificity can be a problem due to false negatives in 5% of the cases. In addition, NAATs can be expensive and time consuming (29).

2.3 Syphilis

Syphilis is an STI that is caused by the Treponema Pallidum, which is a spirochete bacterium (30, 31).

2.3.1 Epidemiology of Syphilis

2.3.1.1 Worldwide

There were 10.6 million new cases of syphilis in 2008. It has been previously known to happen essentially in people between the age groups of 15 and 40, however, it has been also reported that around 1 million newborn children are born each year with innate syphilis (Figure 4) (32).

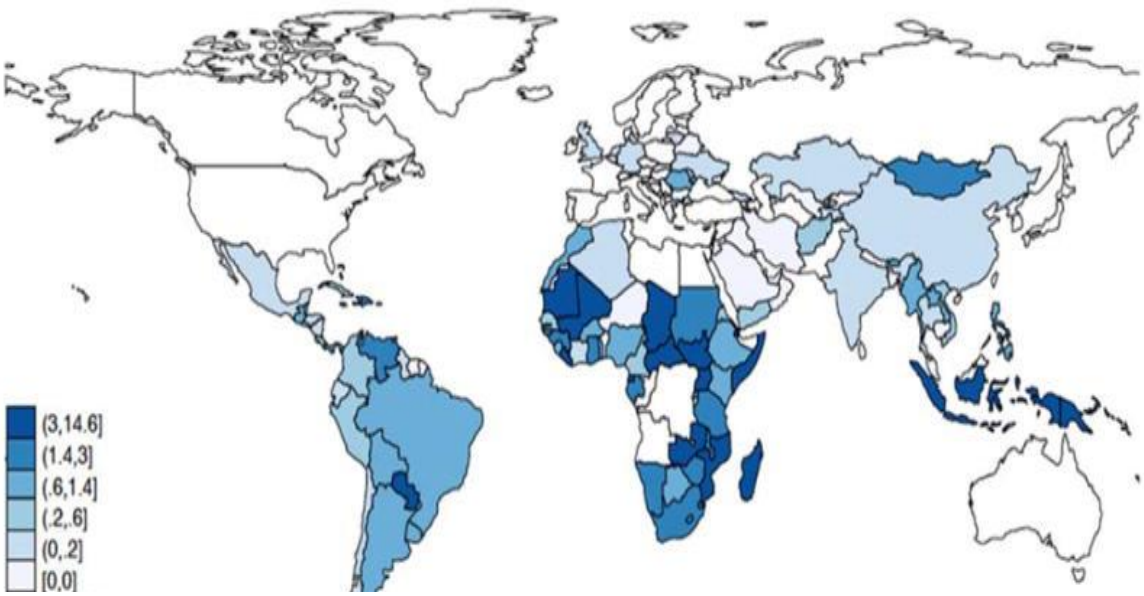

Figure 4 Prevalence of syphilis worldwide

2.3.1.1 Regional

Major differences were recorded between different regions in the world, however the highly affected region that showed consistent infection was Africa (3). Figure 5 summarize the findings of their study.

Content	adjusted odds ratio (AOR)	Percentage	CI
Americas	0.42	95%	0.33-054
Eastern Mediterranean	0.13	95%	0.09-0.19
Europe	0.05	95%	0.03-0.07
South-East Asia	0.21	95%	0.16-0.28
Western Pacific	0.41	95%	0.32-0.53

Figure 5: Percentage of infected Syphilis cases worldwide

2.3.1.1 Qatar

Although no exact statistics are available about the prevalence of syphilis in Qatar, a reported that countries with population making high salaries have a low tendency for the infection as compared to those countries with the majority of its people being either poor or of low income. Therefore, as a high-income country, it can be concluded that Qatar has in general a low prevalence of Syphilis (1).

2.3.2 Classification and Structure of Treponema pallidum

T. Pallidum Subspecies Pallidum is a spirochete with the smallest circular chromosome among prokaryotes that is only of ~1.1 kb in length. As a typical member of the spirochete family, T. pallidum is a gram-negative spiral shape bacterium with both outer and cell membranes with a periplasmic space harboring the flagella (endo flagella). The bacteria possess three to six flagella that give it the ability of great motility

and ability to pass through different membranes. Importantly, the bacteria possess inner membrane lipoproteins that had showed strong reactivity with syphilis patients' serum at different stages of the disease (5).

2.3.3 Pathogenicity of Treponema pallidum

2.3.3.1 Pathogenesis

The clinical course of syphilis is extremely varied and is interrupted by various phases of variable duration as shown in Figure 6. Primary chancre presents as a well-defined ulcer with regular raised or rolled out edges and a clean base. The chancre may be tender due to secondary bacterial infection and may occur in extragenital sites like oral, anorectal, breast and digits. Secondary Syphilis presents with a rash that is non-vesicular, non-pruritic and bilaterally symmetrical (16).

2.3.3.2 Virulence factors

Several virulence components had been identified for T. pallidum's capacity to cause infection. One of which is hemolysin that counts as a crucial factor that lysis cell membranes and helps the bacteria entry (32).

2.3.3.3 Risk factors

Risk factors include different sex practices, which is either not using protective measures or are abnormal practices such as anal sex and sedate utilization (6).

2.3.3.4 Modes of Transmission

The fundamental course of syphilis transmission is sexual contact; however, syphilis can additionally be transmitted through blood, intrinsically, and through non-sexual contact with syphilitic injuries on skin or mucosa. Also, transplacental infection is most likely the major course of fetal contamination (5) as shown in Figure 6.

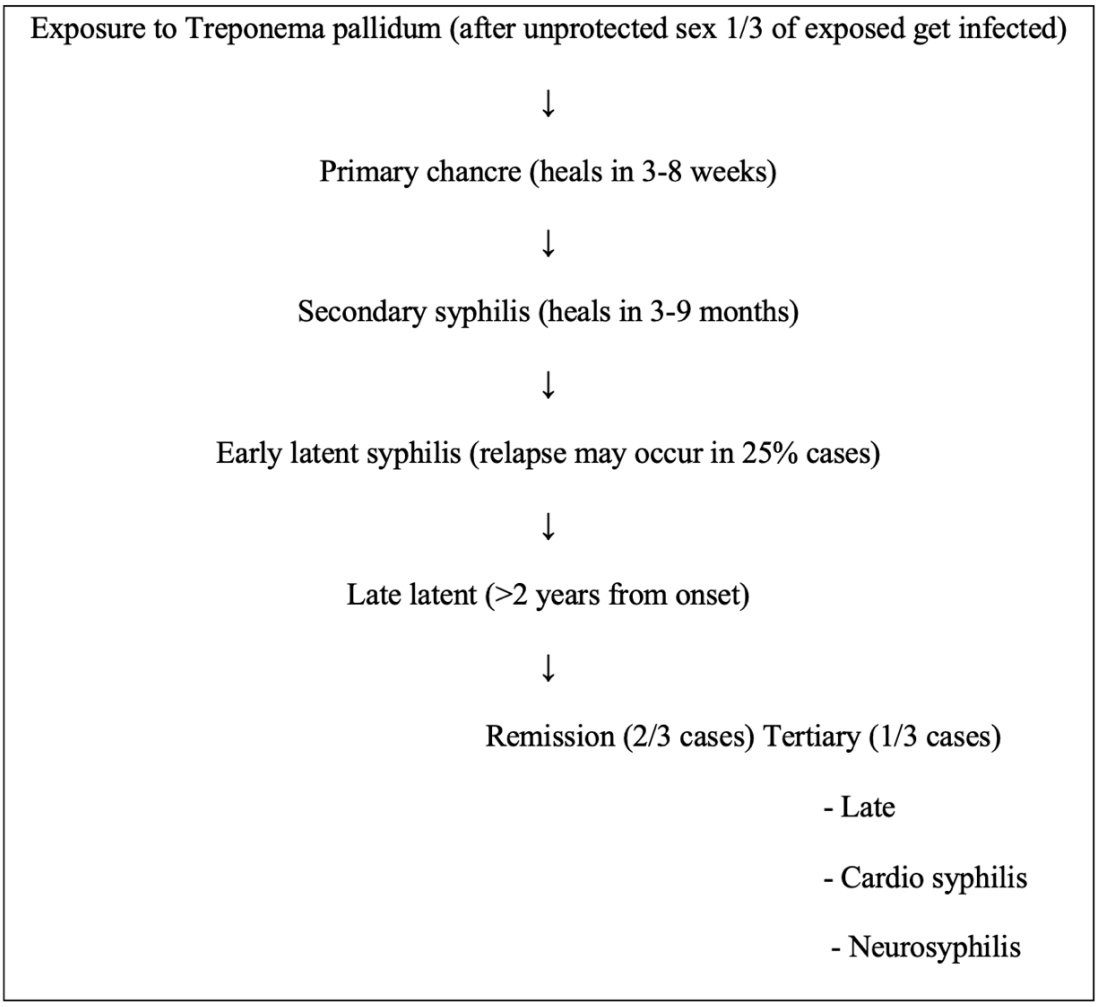

Figure 6 Pathogenesis of syphilis

2.3.3.5 Host genetic determinants

Genome-wide affiliation ponders have distinguished a few hereditary factors/susceptibility loci that are associated with irresistible diseases. Host genetics can determine susceptibility and resistance to infectious disease. Affirmation of the components that decide why few individuals who are uncovered to T. pallidum do not create an infection, is required to get its defensive insusceptibility against T. pallidum (33).

2.3.4 Diagnosis of Syphilis

2.3.4.1 Serological methods

The main method used to diagnose syphilis remains serological testing in spite of the fact that these tests cannot distinguish between syphilis and other treponematoses (5). Serological tests for syphilis consist of two types, non-treponemal and treponemal tests. Non-treponemal tests have high sensitivity but low specificity. Taking into consideration that, viral infections, including HIV and HCV infections can lead to false-positive test results, patients with receptive non-treponemal tests could have corroborative treponemal tests done to confirm the diagnosis (5). Non-treponemal assays include the rapid plasma regain (RPR) and venereal infection investigate research facilities (VDRL) tests, which measure antibodies to lipoidal products. In spite of the fact that non-treponemal tests are viable, cross-reaction happens regularly in case of pregnancy and immune system illnesses such as rheumatoid joint pain which could be seen particularly in early syphilis stages.

Treponemal tests are expensive, time-consuming, and actually troublesome to perform since this bacterium is entirely endobiotic (34). Treponemal tests detect antibodies specifically secreted against T. pallidum proteins. On the other hand, new treponemal tests use exceedingly filtered, recombinant T. pallidum protein antigens,

and polyvalent conjugates that distinguish both IgM- and IgG-class antibodies to syphilis. In fact, it has been shown that treponemal assays are more sensitive in primary syphilis and false positive results are not common (35). It is considered that the non-treponemal tests, treponemal tests and single pathogen rapid detection tests are all basic set of screening tools for syphilis (36).

In a study by Salado (2015), three treponemal tests were used. The first was anti-flagellum determined by a capture ELISA, the second was anti-flagellum decided by a circuitous ELISA, and the third was the Fluorescent Treponemal antibody absorption test done by immunofluorescence microscopy. Combined with specific antibody testing, a Rapid Plasma Regains (RPR) test permits to confirm the conclusion of dynamic disease and to begin treatment. This diminishes the chances of complications and the spread of the disease (5).

The RPR test is used to diagnose syphilis, where it measures antibodies that are present in the blood of syphilis patients, rather than the bacterium that causes the disease. In addition, the RPR test can moreover be utilized to check the advance of treatment for dynamic syphilis. After a course of successful anti-microbial therapy, it is expected to see the titer of antibodies drop, and an RPR test confirms these results. However, syphilis cannot be really ruled out if there are no antibodies, because it takes time for the antibodies to be formed after infection. Thus, shortly after an infection, a test may not yet show positive test for the antibodies, which is often known as an un-true negative. On the other hand, there are many conditions that can cause an un-true positive incorporate HIV, Lyme Illness, Jungle Fever, and Lupus.

Due to the chance of false-positive results appearing, a confirmatory test is usually being done by diluting the samples or by using other assays. For example, there are two types of tests being done; one is a non-treponemal tests like RPR, which is being used

for screening and detection of active disease and for monitoring of the response to treatment. The other test is the treponemal test like TPA which is used as a confirmatory test (37). The use of direct diagnostic methods is limited and that serological tests and treatment calculations contrast from nation to nation (5).

2.3.4.2 Molecular methods

The Polymerase Chain Reaction (PCR) method to detect T. pallidum and hence to diagnose syphilis has been used since 1990 (5). PCR is the most common molecular method used to detect treponemal DNA or antigen in early essential syphilis, early intrinsic syphilis, and neurosyphilis where the application of treponemal serologic tests are constrained. Its affectability in different tissue liquids inspected was 78% comparing with the rabbit infectivity test. Different types of PCR based methods include schedule PCR, Settled PCR, Real-Time PCR, And Multiplex PCR, these are the ones with the quickest improvement, most extensive application, and most profound investigation over the past decade. Schedule PCR and Multiplex PCR can be utilized within the early arrange when the serological response is negative, whereas, settled PCR and real-time PCR can be used for confirmation. They found that multiplex PCR had the highest sensitivity and specificity among the PCR methods mentioned above, almost 100% in all cases. However, as Salado (2015) pointed out, the samples matrix and disease stage can affect the success rate of the diagnostic PCR Moreover, the multiplex PCR test separates syphilis, herpes simplex and chancroid in genital ulcer (38).

Zhou et al. (2019) mentioned the other four types of molecular methods, which are dark-field Microscopy (DFM), Silver Recoloring, Coordinate Fluorescence Immunoassay (DFA), and the Rabbit Infectivity Test. The authors pointed out that the sensitivity of the last test can be sometimes low. DFM is a direct examination of lymph

from the lesion seen beneath the magnifying lens with a dark-field condenser with indirect light (34). It is not expensive and its sensitivity ranges from 74 to 86% and can be higher. Another method is the Fontana-Thibodeau method, where the lymph is smeared on a slide with the addition of silver, which impregnates the treponema wall and makes it visible (39).

In molecular diagnosis, the specimen is collected and then NA is extracted followed by the amplification or hybridization and finally analyzed. Nucleic acid hybridization techniques, amplification techniques, and strain typing, and identification are all types of molecular methods. The target amplification incorporates PCR, real-time PCR and inverts transcription-PCR. Multiplex PCR where both CT and NG are included in a single PCR tube is also used to diagnose syphilis (40).

2.4 Syphilis and HIV co-infection

An interesting study found that HIV co-infection had an effect as it was on the serological reaction in patients with syphilis and a CD4 cell count of <500 cells/μl (41). In this study it has been observed that HIV infected patients are at significantly high risk to get infected with syphilis (IRR = 4.0, 95% CI 2.8–5.9). In fact, HIV coinfection has been illustrated to be vigorously related to syphilis (6). Moreover, Salado (2015) noticed the higher frequency of treatment failures of syphilis among HIV infected patients and concluded that it was most likely due to low immunity in patients infected with HIV (5).

2.5 Medical Commission (MC)

MC in Qatar works under the umbrella of the Ministry of Public Health, and it contributes, on a large scale, to the implementation of the ministry's general strategy in an effective manner. In 2019, MC department screened around 607,601 with an average of 2470 visitors per day.

The MC Vision is to screen all newcomers and residents for the following purposes:

1 Health screening to obtain Medical fitness certificate to apply for a job, travel, or university study in-state

2 Annual Health screening to obtain health certificate for those working either as food handlers, barbers and beauticians, or for individuals in any health club

3 Health screening can also be done for acquiring QCHP license

4 Health screening can be done for changing one's employer

CHAPTER 4: METHODOLOGY

3.1 Ethical consideration

IRB exemption was obtained from Qatar university (QU) and Ministry of Public Health (MOPH) IRB.

3.2 Inclusion and Exclusion Criteria and number of samples

All initial reactive/positive for HIV1-2 Ag/Ab Combo and RPR blood samples that were collected from 1st January 2019 to 31st December 2019, were included and analyzed. We included 595 HIV positive samples and 198 Syphilis positive samples in our study.

3.3 Recruitment Methods

This is a retrospective study. Thus, no patients/applicants were recruited and there was no direct or indirect interaction with a human subject. The study was conducted on existing de-identified testing results in the MC database.

3.4 Confidentiality

The study was conducted on de-identified data and the data was saved in the MC's password-protected database medic system and the students limited access from the section head for de-identified test results information. All samples were bar-coded. In addition, all sample results were stored in the medic system database and password-protected to preserve applicants' identities.

3.5 Data collection and statistical analysis

According to our sample size calculation (based on Stephen Thompson equation, explained in the section below), a total of 595 samples tested positive for HIV1-`2 Ag/Ab Combo collected during the period of January 2019 to December 2019 using Architect from Abbott (USA) and were included in the study. The Replicate ELISA reading is analyzed and used for repeatability and reproducibility purposes. We also included the HIV positive samples confirmed test results by HIV1-2 INNOLIA

confirmatory test and HIV 1 PCR and analyzed for Positive Predictive Value (PPV), true positive, and false positive results.

Moving on to the RPR testing, a total of 198 syphilis reactive samples during the period of January 2019 to December 2019 were included in this study. The results of T. Palladium Antibody (TPA) confirmatory test for these 283 RPR reactive samples were also included and analyzed for PPV, true positive, and false positive results.

All demographical data related the patient's nationality was classified as a continent, patients' gender, and age were also obtained from the MOPH for statistical analysis. The data are saved in the MOPH and kept confidential. No names or any patients' identification was collected. The test measure was calculated based on the confidence level using the Stephen Thompson equation, which was one of the best equations for calculating the sample size. The sample size was calculated based on the confidence level using the Stephen Thompson equation, which is one of the best equations for calculating the sample size. As the confidence level of the sample increases, the sample size increases, and the margin of error will decrease.

$$n = \frac{N \times p(1-p)}{\left[\left[N-1 \times \left(d^2 \div z^2\right)\right] + p(1-p)\right]}$$

N: Population size.

Z: standard value of the significant level (%95) and it equals (1.96).

D: error ratio and it equals (0.05)

P: The ratio of the availability of the property and the neutral and equals 0.50.

3.6 Routine work in MC

3.6.1 HIV Ag/Ab Combo Test for HIV Detection

For the detection of HIV p24 antigen and antibodies, we have used the ARCHITECT HIV Ag/Ab combo assay. This was a qualitative assay that had the advantage to simultaneously detect both p24 antigen and antibody of HIV-1/HIV-2. However, the results of this assay cannot distinguish between the detection of HIV p24 antigen, HIV-1 antibody, or HIV-2 antibody reactivity. This assay was performed according to the manufacturer protocol. Briefly, human serum containing p24 antigens and antibodies were combined with a mixture of assay diluent, washing buffer, and paramagnetic microparticles coated with HIV-1/HIV-2 antigen and HIV p24 mouse monoclonal antibody.

After an incubation period of the samples was washed with the washing buffer to get rid of any unbound complexes. After the final wash cycle, pre-trigger and trigger solution were added to the reaction mixture and the resulting chemiluminescent reaction is measured as relative light units (PLUs). This signal indicated the presence or absence of the antigen or antibodies (exist indirect relationship) in the sample and it was compared to the cut-off signal determined from the assay calibration. The signal to cut-off (S/CO) values determines if the sample was positive or negative to HIV. Samples showing S/CO values ≥ 1.00 were considered reactive (R) to HIV-1 p24 antigen or HIV-1/HIV-2 antibodies, whereas those ≤ 1.00 are non-reactive. Any samples that showed reactivity to HIV antigen and/or antibodies were tested again in duplicates.

3.6.1.1 Interpretation of the Results

1. Specimens with s/co values< 1.00 are considered nonreactive (NR).

2. Specimens with s/co values> 1.00 are considered reactive (R).

ARCHITECT HIV Ag/Ab combo Results: -

· If the first test was reactive it should be rerun in duplicate.

· If both were non-reactive, the result was considered non-reactive.

· If one or both tests were reactive, the result should be considered reactive.

3.6.1.2 Specificity

The Specificity is > or equal 99.5 % with 95% confidence interval.

3.6.1.3 Sensitivity

The sensitivity is as per package insert of the manufacture the Anti-HIV-1sensetivity is 100%, HIV-2 also 100% and HIV group O is 100%. An average responsiveness to HIV-1 p24 Ag of 18.06 Pg./ ml.

3.6.2 - INNOLIA Test (Confirmatory test)

To affirm the nearness of antibodies against (HIV-1) in positive samples, we have used the INNO-LIA™ HIV I/II Score. This assay was a Line Immune Assay (LIA®) that could distinguish between HIV-1/HIV-2, where on a nylon strip discrete lines of HIV-1 and HIV-2 recombinant proteins and engineered peptides are coated (Figure 6). This could detect HIV-1 antibodies to the structural proteins gp120, gp41, p31, p24, and p17, conjointly cross-react with HIV-2 antibodies. In addition, it also could detect Antibodies specific to HIV-2 gp36 and sgp105. This test was performed agreeing to the producer protocol. Briefly, the sample was incubated with the strips mentioned above. A goat antihuman IgG named with Antacid Phosphatase was then included to the antigen-antibody complex. Finally, the enzyme was incubated with the substrate (BCIP/NBT) and the results were recorded. If HIV antibodies were displayed within the test, a dark brown color will

show up. The thickness of the color reflected the number of antibodies present in the sample. Moreover, color development was stopped with sulfuric acid. In case the sample was negative, a background color would appear. All results were compared to the anti-streptavidin and human IgG positive controls. The human IgG positive controls give different color intestines indicating, a range from weak (+) to strong (+++) control lines.

3.6.2.1 Interpretation of the results

1. If there was no line positive and negative, the sample was considered as negative.
2. If there are two or more lines positive and negative, the sample was considered as indeterminate.
3. If a ratio of positive and negative was got on both ENV2 antigen line, the sample was considered as a positive.
4. If a ratio of positive and negative was got on both ENV1 antigen lines, the sample was considered as positive

3.6.2.2 Sensitivity

As a package insert, the sensitivity was 100% for HIV1 and HIV2.

3.6.2.3 Specificity

As a package insert, the calculation of specificity was 96.7%.

Figure 7 INNOLIA test machine.

3.6.3 Diagnosis of HIV in MC

As shown in Figure 8, if the first sample results are negative, then the results will be released without confirmation test. If the first sample result is positive, the same sample will be rerun twice in the same machine. If the first sample results are positive and rerun sample is positive, its consideration as positive. If the first sample result is positive and one of rerun is positive and the other is negative, delete negative and release and consider sample as positive. Transfer the results to doctor for a decision. Call the applicant for new samples (one sample for repeat test and other for PCR). New sample running in other machine and another technician with the same technique. If the result of new sample is positive or negative, we rerun the sample twice. If new sample is positive, rerun sample, however, if negative centrifuge the sample for 10 minutes and rerun twice. If new sample is positive and one rerun sample one positive and the other one is negative, delete the negative and release the positive result. If the new sample positive and rerun is positive, release the results as positive.

3.6.4 COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, version 2.0

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, version 2.0 (v2.0) is an in-vitro nucleic acid amplification test that is used for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma using the COBAS® AmpliPrep Instrument for automated specimen processing and the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer for automated amplification and detection.

The test can help in quantitating HIV-1 RNA over the range of 20 - 10,000,000 copies/mL (33 to 1.67 x 107 International Units [IU]/mL). In addition, one copy of HIV-1 RNA is equivalent to 1.67 IU based on the WHO 1st International Standard for HIV-1 RNA for Nucleic Acid-Based Techniques (NAT) (NIBSC 97/656) 36.

The test is generally intended for use in combination with clinical manifestation and other laboratory markers of disease progress that are used for the clinical management of HIV-1 group M and HIV-1 group O infected patients. Moreover, the test can be used to evaluate patient prognosis by determining the baseline HIV-1 RNA level or to screen the effects of antiretroviral therapy by measuring deviations in EDTA plasma HIV-1 RNA levels during the period of antiretroviral treatment. Furthermore, COBAS AmpliPrep/COBAS TaqMan HIV-1 Test v2.0 is not proposed to be used as a screening test for the presence of HIV-1 in blood or blood products or as a diagnostic test to verify the existence of HIV-1 infection.

3.6.4.1 Principles of the Procedure

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 is actually a nucleic acid amplification test used for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 is based on three major processes: (1) specimen preparation to separate HIV-1 RNA; (2) reverse transcription of the target RNA to produce complementary DNA (cDNA), and (3) simultaneous PCR amplification of target cDNA and the identification of cleaved dual-labeled oligonucleotide detection probe that is specific to the target.

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 authorizes automated specimen preparation which is followed by automated reverse transcription, PCR amplification and detection of HIV-1 target RNA and HIV-1 Quantitation Standard (QS) Armored RNA. The Master Mix reagent contains primers and probes that act specific for both HIV-1 RNA and HIV-1 QS RNA. The Master Mix has been established to guarantee equivalent quantitation of group M subtypes of HIV-1 and of HIV-1 group O.

In addition, the detection of amplified DNA is done using target-specific and QS-specific dual-labeled oligonucleotide probes that allows for independent identification of HIV-1 amplicon and HIV-1 QS amplicon. The quantitation of HIV-1 viral RNA is accomplished using the HIV-1 QS. It is used to compensate for effects of inhibition and controls the preparation and amplification processes, allowing a more accurate quantitation of HIV-1 RNA in each specimen. The HIV-1 QS is a non-infectious Armored RNA construct that contains HIV sequences with same primer binding sites as the HIV-1 target RNA and a unique probe binding region.

It, therefore, permits HIV-1 QS amplicon to be differentiated from HIV-1 target

amplicon. The HIV-1 QS is added to each specimen at a known copy number and is run through subsequent steps of specimen preparation, reverse transcription, simultaneous PCR amplification and detection of cleaved dual-labeled oligonucleotide detection probes. The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer is used to calculate the HIV-1 RNA concentration in the test specimens by matching the HIV-1 signal to the HIV-1 QS signal for each specimen and control.

3.6.4.2 Specificity

The specificity of the test was calculated based on a total sample size of 566 HIV-negative sample. A total of 562 (99.3%) samples tested true negative while 4 (0.7%) samples were false positive.

The specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 was determined with two reagent lots by analysis of HIV-1-negative EDTA plasma specimens from blood donors. A total of 660 individual EDTA plasma specimens indicated valid results where all the results were negative for HIV-1 RNA in the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0. Based on these results, the specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 came out to be 100% (one-sided lower 95% confidence limit: ≥ 99.6)

The clinical sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 was defined as the percentage of evaluable HIV-1-positive subjects who obtained a positive COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0.

3.6.4.3 Sensitivity

The sensitivity of the test was calculated based on a total sample size of 418 HIV-1-positive sample. A total of 418 (100%) samples tested true positive while no sample (0%) tested negative. This showed a 100%specificity of the test.

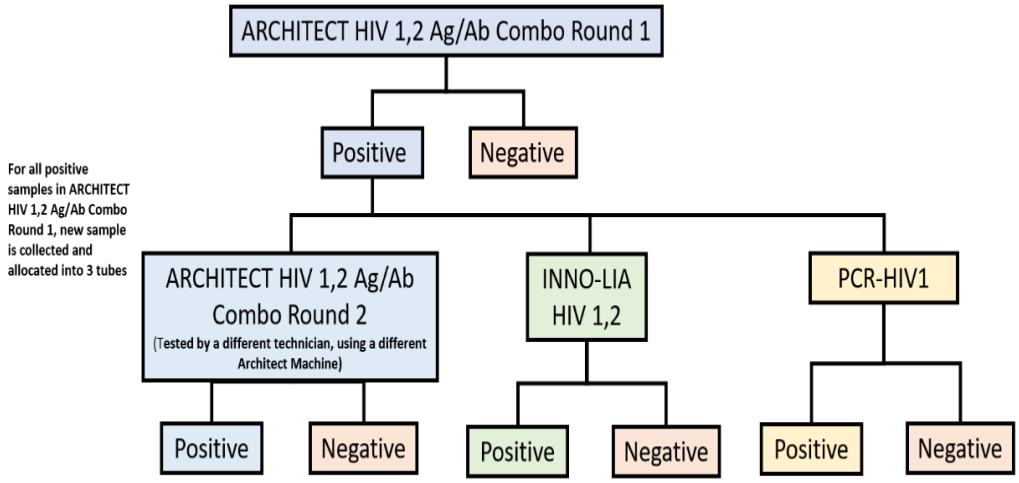

Figure 8 A chart summary of HIV diagnosis workflow.

3.6.5 Rapid Plasma Reagin RPR Test (Omega diagnostics of syphilis)

For the detection of syphilis, we used the Rapid Plasma Reagin (RPR) card test. This was qualitative and semi-quantitative non-treponemal flocculation assay used to detect regain antibodies in serum of patient samples. The principle of this assay was based on the presence of carbon particles in the antigen, which when bind to antibodies improved the visual reading of the flocculation reaction. This reaction could be observed macroscopically as black clumps when the interaction occurs cholesterol/ cardiolipin/ lecithin in the reagent and the reagin antibodies in the sample. The formation of these clumps is considered a positive reaction to syphilis, whereas a nonreactive negative result is indicated by no visual flocculation.

This assay was performed according to the manufacturer protocol. Briefly, 50 µl of patient sample was placed and spread accurately to cover a defined circle in the RPR Test Card. Then 16 µl of the antigen (provided by the manufacturer) was added to the sample and mixed by rotation 100 rpm for 8 minutes on an automatic rotator.

Antigen-antibody interaction to detect black clumps was then observed visually under the light. Positive samples were then subjected to semiquantitative analysis, which was done as follows: A two-fold serial dilution of the patient serum sample was done using isotonic saline. Then 16µl of the antigen was be added to each dilution and the assay was completed as mentioned above. Positive and negative controls supplied by the manufacturer were also added and tested each test.

3.6.5.1 Results and interpretation

As shown in Figure 8 and 9, Manual test (Use carbon coated antigen). If the sample result is negative, consider as negative. However, if the sample result is positive, make a dilution to find the titer. Make a dilution by using isotonic saline 0.9% NaCl. Release the results and send it to the doctor. Doctor make the decision to send the positive sample to HMC for confirmatory test.

Qualitative Method:

- Positive (reactive): Medium and large aggregates or clumps.
- Weak positive (reactive): Finely dispersed aggregates.
- Negative (non-reactive): No aggregates visible with smooth grey appearance.

Semi-Quantitative Method:

The titer of the antibody was calculated based on the last dilution showing a reactive result as compared to the positive control.

Figure 9 Rapid Plasma *Reagin RPR Test (RRR)*

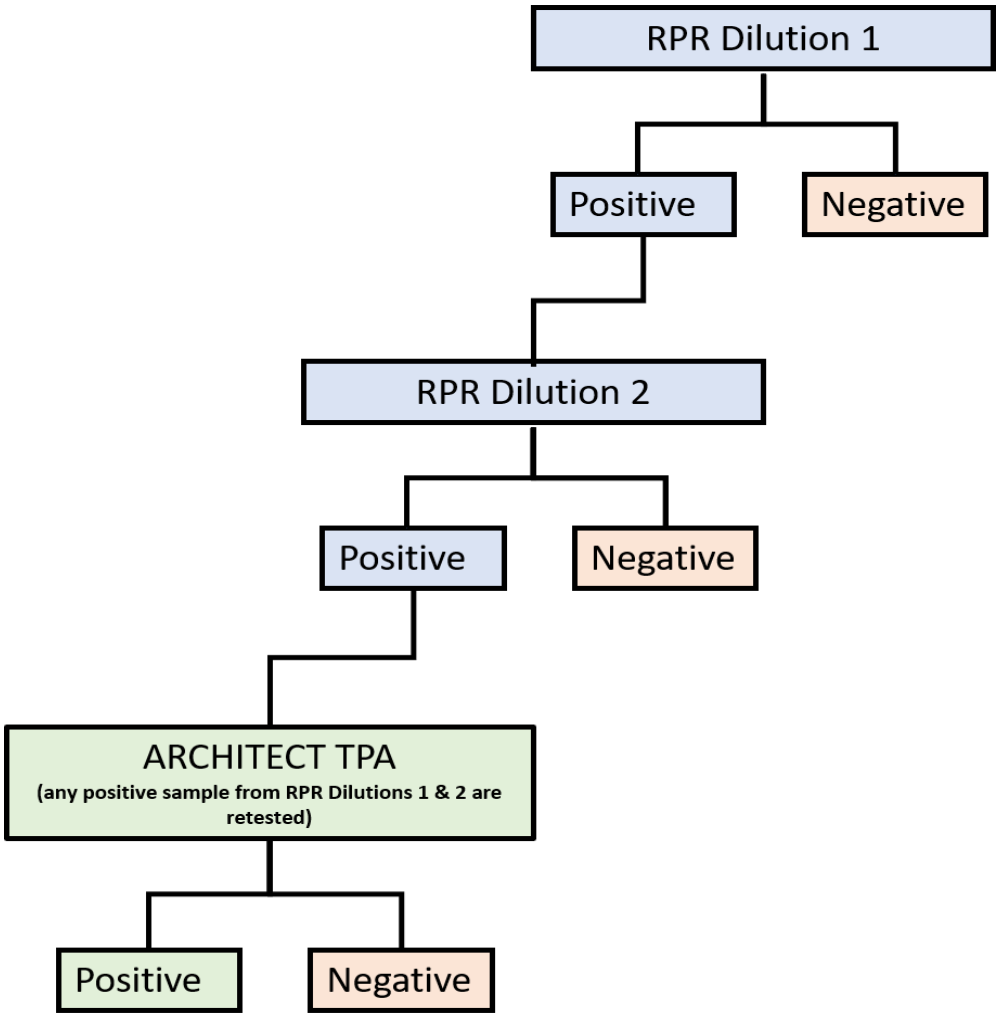

Figure 10 A chart summary of syphilis diagnosis workflow

3.7 Routine Work in HMC:

3.7.1 TPHA Bio rad

TPHA kits are intended for usage of the qualitative and semiquantitative detection of antibodies to Treponema pallidum that could be present in human serum and plasma. The product may be utilized for the screening of blood donors, and also to assist in the diagnosis of patients where syphilis infection is suspected.

Principles of the Procedure

TPHA kits use preserved avian erythrocytes coated with antigens of T. pallidum (Nichols strain), which will bind with specific antibody present in patient's serum or plasma. The cells are suspended in a medium containing component that helps in eliminating non - specific reactions. Positive reactions are usually demonstrated by agglutination of the cells, negative reactions are demonstrated by the settling of the cells to a button or small ring.

Even though these kits are meant for use primarily as qualitative tests, antibody levels can be titrated by doubling dilution as well.

Agglutination patterns can be interpreted through naked eye or through a plate-reader which can read agglutination patterns Figure 10. A contact to the concerned company can be made in case of adaptations and special procedures.

In general, there are five potential results of any tested sample. The sample is considered strong positive when full cell pattern covers the bottom of the well with negative result in the control. A weak positive result is when the pattern covers 1/3 of the well bottom and control cells tested negative. Equivocal samples have cell patterns that shows distinctly open centers. Non-specific reaction will have both test and control cells positive reaction (Figure 10).

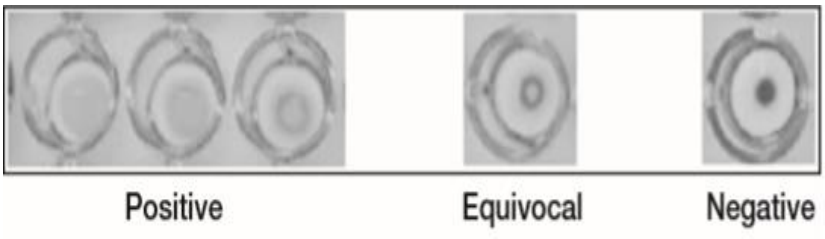

Figure 11 Agglutination patterns

3.7.1.1 Specificity:

A total of 5032 samples from blood donors, prospectively gathered at 2 different sites, were studied. The samples were either serum (3626), EDTA K2 (539) or Lithium Heparin plasma (867) that were tested in a period of less than 7 days after sampling. They were then compared to the screening assay used in the laboratory.

The total specificity on the blood bank population equates to 99.72% (5017/5031) with a 95% confidence interval of 99.53% to 99.85%. In terms of the 14 false positive samples, 11 were discovered to be repeated equivocal.

A retrospective study was also carried out on 201 frozen samples from patients who belonged to a Sexually Transmitted Disease Center or vendors. The samples were found negative for syphilis. Specificity on these samples was found to be as 99.5% (200/201) with a 95% confidence interval at 97.3% to 100.0%.

3.7.1.2 Sensitivity:

The sensitivity study was studied on 435 retrospective frozen serum samples from the laboratory of Sexually Transmitted Disease Center, or from frozen samples from vendors. These samples were described as positive by immunoassays, FTA assay, RPR/ VDRL assay or TPHA assays according to their sources or origins. All the samples were first tested with the utilization of CE mark TPHA assay and later with the Bio-Rad TPHA 500 assay (72504). Sensitivity on this population was found to be at 100% (435/435) with a 95% confidence interval at 99.2% to 100.0%.

CHAPTER 5: RESULTS

5.1 Comparison between different methods used in HIV diagnosis:

Retrospective data had been collected from a total of 585,587 individuals who visited the MC in the period between January 1, 2019 and December 31, 2019. Among the total of 585,587 individuals, we found 595 positive samples. This data was obtained from two different rounds of ARCHITECT assay as well as from INNO-LIA HIV and PCR that were used as confirmatory tests. The ARCHITECT and INNO-LIA HIV assays were done at the MC laboratory, whereas PCR was done at HMC. It is worth noting that the ARCHITECT assay was performed in two different ARCHITECT machines (analyzers A and B) and done by two different technicians, hence, named rounds 1 and 2, respectively. The results obtained from all these assays were analyzed and compared to each other to test the reproducibility and cost effectiveness of this diagnostic protocol.

In the initial HIV screening (round 1), the ARCHITECT assay identified all the 595 tested samples positive to HIV. To confirm such reactivity, blood samples were recollected from all positive cases and round 2 of ARCHITECT assay was done using analyzer B. From this analysis, one sample was negative to HIV and 594 were HIV positive as shown in Table 2.

The results were then compared to those obtained from the first round of screening (ARCHITECT analyzer A; round 1) using percent agreement analysis. The two rounds showed a 99.83% agreement (overall and positive percent agreements; 95% CI, 99.05-99.97%) (Table 6). Next, it was important to compare the results obtained from round 2 analysis with those obtained from INNO-LIA HIV and PCR. The INNO-LIA HIV assay correctly identified 173 HIV positives, 371 HIV negatives, and 50 Indeterminate (Table 3). Indeterminate samples mean that there was a possibility of the presence or absence of HIV-specific IgG antibodies.

The overall percent agreement (95% CI) between the results obtained from INNO-LIA HIV and round 2 ARCHITECT analyzer B was then calculated. Even though, low overall percent agreement of 31.8% (28.3%-35.83%) was observed between the two assays. the positive percent agreement (95% CI; 97.83%-100%) was 100% as shown in Table 6. To ensure these results indicate the true positive cases, we calculated the positive predictive value (PPV). Similar to the results obtained for the overall percent agreement of the ARCHITECT analyzer B and INNO-LIA, the PPV was 31.8% (95% CI; 28.3%-35.83%) (Table 6).

Comparable results were obtained with the PCR analysis that showed 157 HIV positive samples and 429 negative ones (Table 4), with an overall percent agreement of 26.79% (95% CI; 23.36%-30.52%) and positive percent agreement of 100% (95% CI; 97.61%-100%) (Table 6). As expected, the PPV value was similar to the overall percent agreement indicating the true positive HIV cases to be 26.79% (95% CI: 23.36% - 30.52%).

Finally, we compared the results obtained from the two confirmatory tests (PCR and INNOLIA). Both assays showed the same results in a total of 525 samples, where 155 were HIV reactive, 370 non-reactive, and one was indeterminate. However, there was discrepancy in 49 samples that showed reactivity in INNOLIA but not in PCR (Table 5). Interestingly, the agreement between the two assays was high as it reached 97.77% (95% CI; 96.14%-98.72%) and 93.94% (95% CI; 89.21%-96.68%) in overall percent agreement and positive percent agreement, respectively (Table 6).

Table 1 Comparison between ARCHITECT anti-HIV round 1 and round 2

ARCHITECT HIV Ag/AB Combo Round2 / ARCHITECT HIV Ag/AB Combo Round1	Positive	Negative
Positive	594	1
Negative	0	0

Table 2 Comparison between ARCHITECT anti-HIV *round 2 and INNO-LIA HIV*

INNO-LIA HIV / ARCHITECT HIV Ag/AB Combo Round2	Positive	Negative	Indetermined
Positive	173	371	50
Negative	0	0	0

Table 3 *Comparison between* ARCHITECT anti-HIV round 2 and PCR

PCR / ARCHITECT HIV Ag/AB Combo Round2	Positive	Negative
Positive	157	429
Negative	0	0

Table 4 Comparison between INNO-LIA HCV and PCR

INNO-LIA HIV / PCR	Positive	Negative	Indetermined
Positive	155	1	1
Negative	10	370	49

Table 5 The performance agreement of HIV between different methods

	Overall Percent Agreement % (95% CI)	Positive Percent Agreement % (95% CI)	Negative Percent Agreement % (95% CI)	Positive Predictive Value % (95% CI)
ARCHITECT HIV Ag/AB Combo Round1 & ARCHITECT HIV Ag/AB Combo Round2	594/595 99.83% (99.05%-99.97%)	594/594 100% (99.36%-100%)	N/A	N/A
ARCHITECT HIV Ag/AB Combo Round2 & PCR	157/586 26.79% (23.36%-30.52%)	157/157 100% (97.61%-100%)	N/A	157/586 26.79% (23.36%-30.52%)
ARCHITECT HIV Ag/AB Combo Round2 & Lnno-Lia HIV	173/544 31.80% (28.03%-35.83%)	173/173 100% (97.83%-100%)	N/A	173/544 31.80% (28.03%-35.83%)
PCR & Inno-Lia HIV	525/537 97.77% (96.14%-98.72%)	155/165 93.94% (89.21%-96.68%)	370/371 99.73% (98.49%-99.95%)	155/156 99.36% (96.46%-99.89%)
Order of reference standard: INNO-LIA HIV 1,2 > PCR-H1V1 > ARCHITECT HIV 1,2 Ag/Ab Combo Round 2 > ARCHITECT				

5.2 Comparison between different methods used in syphilis diagnosis:

For syphilis analysis, data was obtained from the RPR test performed on 97,298 blood samples (collected between January 1, 2019 and December 31, 2019) to screen for the disease. Among the total of 97,298 individuals, we found 198 positive samples. The test was done in two different dilutions (1 and 2) and by two different technicians and the Medical Health Commission laboratory. The results from the two dilutions were compared and, in both dilutions, all the 198 samples tested were reactive to syphilis (Table 7). Indeed, this full agreement between both dilutions was confirmed with the agreement analysis that showed an overall and positive percent agreement of 100% (95% CI; 99.72-100%) (Table 9).

The results obtained from the RPR was further confirmed by TBA ELISA (Done in Hamad Medical Corporation). We also collected the data produced from this confirmatory test and compared it to those of RPR dilution 2. In contrast to the results obtained from RPR dilution, TBA ELISA identified 72 syphilis positive samples and 125 negative samples (Table 8). In line with this, the overall percent agreement between the two assays was 36.55% (95% CI; 30.14%-43.47%), whereas the positive percent agreement was 100% (94.93%-100%) (Table 9). To ensure true positive cases, the PPV was calculated. Similar to the results obtained for the overall percent agreement of the RPR dilution 2 and TBA ELISA, the PPV was 36.55% (30.14%-43.47%) (Table 9).

Table 6 Comparison between RPR dilution 1 and 2

RPR Dilution 2 / RPR Dilution 1	Positive	Negative
Positive	198	0
Negative	0	0

Table 7 Comparison between RPR dilution 2 and ELISA

TPA ELISA / RPR Dilution 2	Positive	Negative
Positive	72	125
Negative	0	0

Table 8 The performance agreement of Syphilis between different methods

	Overall Percent Agreement % (95% CI)	Positive Percent Agreement % (95% CI)	Negative Percent Agreement % (95% CI)	Positive Predictive Value % (95% CI)
RPR Dilution 1 & RPR Dilution 2	198/198 100% (98.10%-100%)	198/198 100% (98.10%-100%)	N/A	N/A
RPR Dilution 2 & TPA ELISA	72/197 36.55% (30.14%-43.47%)	72/72 100% (94.93%-100%)	N/A	72/197 36.55% (30.14%-43.47%)

CHAPTER 6: DISCUSSION

In the present study, we were able to show that Abbott ARCHITECT HIV Ag/Ab Combo assay could be used efficiently in the MC for HIV screening. The assay performs with high sensitivity and specificity with the two rounds repeats done on two different analyzers and by two different technicians. This high sensitivity and specificity indicate that the protocol used in MC is highly accurate in performing the assay as an initial screening for HIV. This is an extremely important finding because it significantly decreases the chances of false positives or false negatives, which in-turn decrease the number of people who can potentially receive wrong information about their health status. False reactive test results can also occur due to a degree of cross-reactivity with other pathogens. On the other hand, testing an individual at early stage of HIV infection may results in false negative diagnosis. This should be also prevented to decease the widespread of the infection in the country. Our results are supported by the data obtained from different other ARCHITECT evaluations that were done on similar or higher numbers of samples from different countries (42-44)

Our comparison between ARCHITECT results and the PCR results showed an overall percent agreement of 26.79%, indicating a very low agreement in the results obtained from the two assays. The PCR assay showed a significantly lower number of positive HIV cases compared to ARCHITECT (Table 5). This may be due to the inability of PCR to identify some HIV infections, such as those on antiviral therapy. Indeed, reports from other laboratories using Nucleic acid amplification tests (NAAT) had shown that this technique might have several limitations in HIV screening as it is expensive and missing the detection of some HIV cases (19, 45). Moreover, the majority of the current PCR assays are designed to detect Subtype B HIV-1 but are not optimal to detect other types of HIV.

Furthermore, seroconversion panels for non-HIV-1 subtypes and HIV-2 are not available (46). In addition, it is known that HIV subtypes differ according to the geographical distribution, where subtype C is the predominant type in India, Bangladesh, and Nepal (47, 48). Besides, many studies' results supported our findings and showed high sensitivity and specificity of the ARCHITECT combination assay. For instance, a study aimed to evaluate the performance of ARCHITECT on well-characterized specimens. Results showed that the initial ARCHITECT testing sensitivity was 99.94% and specificity was 98.78% (49). Another study showed that the specificity of the ARCHITECT was 99.5% and sensitivity 94% (50). Yet, it worth mention that Architect could generate a relatively high number of false positive results. In addition, newly infected individuals might be misdiagnosed as false negative (51).Therefore, detecting HIV antigens could shorten the window period and decrease the false negative results (52).Given that the Medical Health Commission is the main hub to screen individuals from different parts of the globe, with the majority coming from South Asian countries, makes it essential to carry tools that can detect all subtypes of HIV. Therefore, it is difficult to apply PCR as a specific tool to screen for HIV, and the ARCHITECT will remain the preferable technique used for massive screening of HIV. Future studies are needed to assess more the agreement between the two assays using a different set of primers that can detect different HIV subtypes.

Improving the diagnostic tools for syphilis becomes an important concept to advance the level of care for STD patients. Different reports had shown frequent discrepancies in the results of the RPR test indicating the urgent need for regular quality control and enhancing the standards of the test (53). The inconsistent RPR titers between different laboratories raise doubts if this assay is the best to be used in syphilis screening as false results affect the on-the-treatment decision as well as the disease

outcomes. Conversely, our results showed 100% overall and positive percent agreements between two RPR tests done by two different dilutions and performed by two different technicians (Table 9). Such consistency in the results indicates the validity of RPR at the Medical Health Commission and its crucial role as a reliable test. The differences in RPR results that have been seen in different laboratories could be due to a number of reasons such as storage of sample, freezing and thawing effects, and RPR test cards. There are many different RPR test cards available (BD Macro-Vue™ RPR and Carbon, Cypress Diagnostics) and each laboratory is using different cards; it could be the case that even within the same laboratory different cards are used. At the Medical Health Commission, all the test parameters had always been very consistent, which help to obtain such reproducible results.

Our results demonstrated significant differences in the number of positive samples detected by RPR and ELISA, where the two methods showed a low overall percent agreement of 36.55% (Table 9). In agreement with other results, other studies had shown a non-statistically significant differences between the sensitivity of ELISA and the RPR test (54). It showed that ELISA is sensitive in all stages of the disease, while other studies showed that the assay is of low sensitivity during the early syphilis phase (29, 55). Furthermore, it has been previously shown that the ELISA sensitivity in detecting syphilis ranges from 45-100%. This wide range of sensitivity may be due to the type of ELISA used (56), stage of the disease (57), or laboratory environment and tools (58-61). Indeed, in our study, ELISA had been done in another institute (Hamad Medical Corporation), which may be the reason behind these differences observed in the results obtained from both methods.

Limitation:

The main limitation of the study is that we did not have the diagnostic test results of HIV negative samples, so we could not calculate the specificity and sensitivity of the diagnostic test used. Therefore, we were not able to confirm the negative cases.

Recommendations:

Since our data showed that there is a 100% agreement between the Architect result of the first and fresh repeat samples, we recommend not asking for repeat samples in order to save cost and time. Also, our data showed that there 100% agreement between the first run of RPR in comparison to the re-run by another lab technologist, therefore we recommend stopping rerun the RPR test.

CHAPTER 7: CONCLUSION

In conclusion, the techniques used in the Medical Health Commission are reliable and reproducible and efficient for the screening of STDs. Nevertheless, further studies are needed to test the agreement of these tests with other confirmatory tests done at Hamad Medical Corporation. Also, it is important to determine the cost-effectiveness of the implementation of these confirmatory tests in the Medical Health Commission laboratory to test the performance of these assays under the same environment and by the same technicians.

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Appendix A

January 26, 2020

Exempt Research Certificate

Dear Applicant,

The Health Research Governance Department at the Ministry of Public health (MoPH) has reviewed the research proposal entitled "*Performance evaluation of the Architect automated immunoassay for diagnosis of HIV and the performance evaluation of the Rapid Plasma Regain test for screening for Syphilis*". The Principal Investigator, Ms. Raniya Abdulla Rashid Al-Buainain, confirmed that there will be no collection of identifiable information. Upon review, the project has been categorized as **exempt research under category (3):** Research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified.

However, please note that in accordance with MoPH policy, the regulations state that "research involving...interview procedures...{**is exempt from this policy**} unless (1) information obtained is recorded in such a matter that human subject can be identified directly or through identifiers linked to the subject **and** (2) disclosure of the human subject responses outside the research could reasonably place the subjects at risk of criminal or civil liability, or be damaging to the subjects' financial standing, employability, or reputation". Under conditions mentioned in (1) and (2), the proposal must be reviewed by an Institutional Review Board Committee.

If we can be of further assistance, please contact us at 974-4407-0363 or via email at IRB@moph.gov.qa

Sincerely,

Nardin Fallouch
on behalf of

Dr. Eman Sadoun
Manager, Health Research Governance
Ministry of Public Health
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Appendix B

جامعة قطر
QATAR UNIVERSITY

Qatar University Institutional Review Board **QU-IRB**
QU-IRB Registration: IRB-QU-2020-006, QU-IRB, Assurance: IRB-A-QU-2019-0009

February 26th, 2020

Dr. Gheyath Nasrallah
College of Health Sciences
Qatar University
Phone: +974 4403 4817
Email: gheyath.nasrallah@qu.edu.qa

Dear Dr. Gheyath Nasrallah,

Sub.: Research Ethics Review Exemption
Project Title: "Performance evaluation of Architect automated immunoassay for diagnoses of HIV and the performance evaluation or rapid plasma regain test for screening for syphilis"

We would like to inform you that your application along with the supporting documents provided for the above project, has been reviewed by the QU-IRB, and having met all the requirements, has been granted research ethics **Exemption** based on the following category(ies) listed in the Policies, Regulations and Guidelines provided by MoPH for Research Involving Human Subjects:

Exemption Category 3: Research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified.

Documents reviewed: QU-IRB Application Human Subject-V3_Bilingual_Feb2019 (002), Raniya QU-IRB Checklist, Research Proposal (Ranyia proposal), MoPH Approval letter (Ms. Raniya Abdulla Rashid Al-Buainain Exempt research certificate), Data Collection Sheet, QU-IRB Review Forms, responses to IRB queries and updated documents.

Note: Please note that the investigator is responsible for compliance to any additional ethical procedures or regulations required at the Medical Commission and/or its designated laboratories.

Please note that exempted projects do not require renewal; however, any changes/modifications to the original submitted protocol should be reported to the committee to seek approval prior to continuation.

Your Research Ethics Approval Number is: **QU-IRB 1242-E/20**. Kindly refer to this number in all your future correspondence pertaining to this project. In addition, please submit a closure report to QU-IRB upon completion of the project.

Best wishes,
Dr. Ahmed Awaisu
Chairperson, QU-IRB

جامعة قطر
QATAR UNIVERSITY
Institutional Review Board
(IRB)
Office Of Academic Research