QATAR UNIVERSITY

COLLEGE OF HEALTH SCIENCES

PERFORMANCE EVALUATION FOR THE HBVAND HCV DETECTION PROTOCOLS

THAT ARE USED BY THE MEDICAL COMMISSION DIAGNOSTIC LABORATORY

IN QATAR

BY

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ABSTRACT

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Title: PERFORMANCE EVALUATION FOR THE HBVAND HCV DETECTION PROTOCOLS THAT ARE USED BY THE MEDICAL COMMISSION DIAGNOSTIC LABORATORY IN QATAR

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Background: Viral Hepatitis is regarded as a serious concern to human wellbeing, affecting millions of individuals yearly. The leading cause of this disease is the infection of Hepatitis A, B, C, D, and E. Consequently, leading to several complications, including liver cirrhosis and tumor formation. The World Health Organization (WHO) estimated that one in three people around the globe have been infected by either HBV or HCV.

Objectives: We aim to achieve the highest level of accuracy and reliability of our testing, and to evaluate the primary and secondary tests used to diagnose HBV and HCV at the medical commission of Qatar.

Methods: This retrospective study was conducted based on recorded laboratory results for hepatitis B (n=1061) and hepatitis C (n=1399) during the period of January 1, 2019, to December 31, 2019. The obtained data for hepatitis B included ARCHITECT immunoassay; while the hepatitis C data included ARCHITECT immunoassay, INNOLIA (line immune Assay), and Polymerase chain reaction (PCR).

Results: A total of 334.313 HBV and 334.312 HCV cases were analyzed in Medical commission during 2019. Data analysis was done on a total of 1061 and 1399 positive individuals who were previously screened for HBV and HCV, respectively. Our HBV

results showed high reproducibility and reliability of the Architect assay as indicated by the 100% overall percent agreement and positive percent agreements between the different runs of the test as well as different Architect analyzers. Regarding HCV results, our analysis indicated that Architect did not show a high percent agreement with those of PCR (overall percent agreement and PPV was 5.77%).

Conclusion: The study illustrated that the agreement percentage was the highest for Ant-HBS and Anti-HCV with excellent performance for the Architect system. It demonstrated high specificity and 99.91% overall agreement with different Architect analyzers. and 100% positive predictive agreement. Yet, Architect had 67.20% overall agreement between HBsAg round 2 final positive and Architect neutralization test. Regarding HCV testing, the Architect demonstr ated high specificity with a 100% overall agreement between round 1 and round 2 of anti-HCV testing. Besides, it showed a 100% positive predictive agreement between Architect anti-HCV round 2 and PCR as well as Architect anti-HCV round 2 and INNO-LIA HCV. Yet, the overall agreement were 5.77% and 28.32%, respectively. In addition, the evaluation of INNO-LIA HCV and PCR showed a 100% positive predictive value with 79.22% overall agreement. The results showed that the procedures of the Medical commission testing platform gives precise and reproducible results. Therefore, there is no advantage gained from repeating Architect analysis, and it is recommended to healthcare facilities in Qatar to use Architect only one time. In this manner, we would be able to successfully save our limited resources while reducing cost and time for future testing procedures.

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 grateful my Supervisor and my committee members for their help and effective comments

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TABLE OF ABBREVIATIONS

Abbreviation	Meaning
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HAV	Hepatitis A virus
HDV	Hepatitis D virus
HBsAg	Hepatitis B surface antigen
HEV	Hepatitis E virus
HCC	Hepatocellular carcinoma
PCR	Polymerase Chain Reaction
RT-PCR	Real-Time Polymerase Chain Reaction
EIAs	Enzyme Immunoassays
ELISA	Enzyme-Linked Immunosorbent Assay
NAT	Nucleic Acid Test
SVR	Sustained Virologic Response
POC	Point Of Care
WHO	World Health Organization
IRES	Inner Ribosome Section Location
LDL	Low-Density Lipoprotein
LDLr	Low-Density Lipoprotein Receptor
CTLA4	Cytotoxic Lymphocyte-Associated Antigen4
HNF	Hepatocyte Nuclear Factors
ORFs	Open Perusing Outlines
ECLIA	Electrochemiluminescence Technology
CLIA	Chemiluminescence
RLU	Reactive light unit
CMIA	Chemiluminescent Microparticle Immunoassay

CHAPTER 1: INTRODUCTION

Viral hepatitis is a major public health concern, affecting millions of individuals globally yearly. The disease, which is caused by a variety of viruses (A, B, C, D, E, and others), leads to several complications including liver cirrhosis that might end up with the formation of tumors in the infected liver. Particularly, the formation of hepatocellular carcinoma (HCC) leads to a high number of fatalities among HCV followed by HBV- infected patients (1). The two most well-known viruses that lead to such persistent liver illness around the world are hepatitis B and C infection (HBV and HCV) (2). There is about three percent of people all over the world who have been infected with HCV; it should, however, be noted that the prevalence of hepatitis C virus depends on countries with infection rates of about 20 % in countries where the disease is endemic (3).

Hepatitis C virus has become a model virus that determines a new model in virology, immunology, and science (4). The issue proceeds to trigger and challenge the wellbeing, due to the presence of several different complications that are undiscovered with different cases of HCV infections. The most common of these complications are liver cirrhosis and hepatocellular carcinoma (5). The development of HBV and its outcomes depends on many factors: genetics, other comorbidities, immune status of the patients, and others (6).

There are currently several different methods in existence for the diagnosis of HBV and HCV. Examples include serological methods, molecular methods, or novel diagnostic tools. Serological methods are widely utilized as one of the essential ways of a testing method to ensure the presence of HCV infection by testing for antibody responses against Hepatitis C. A signal-to-cut-off value (S/CO) can be used in this type of testing (7).

Another alternative method proposed to diagnose the HBV or HCV infection was molecular methods. This method was used to replace serological methods due to its inability of detecting active viruses especially in the early phase of infection(8). One of the advantages of using such a method is achieving a high specificity and high sensitivity as compared to serological methods and it has known to become as economically affordable for low-income nations with high prevalence rates of such infection(8).

Also, recently, several novel diagnostic tools have been in place that include non-immunological Point of Care (POC) tests that utilize entire blood, serum, or plasma collected by venipuncture. Similarly, important advances have been made in the diagnostic field such as immunochromatographic strips, flow-through tests, and tests based on solid-phase measures (9).

All the above information reflects how vital it is to diagnose such infections to start treatment and decrease the burden and complications of the infectious disease. Serological tests to detect viral antigens or antibodies raised against HCV or HBV are known to be the most common tests used to diagnosis infections (2). Moreover, subjective and quantitative HCV atomic tests are utilized in cases of severe and chronic disease (8). Moreover, some novel methods for diagnosis can also be effectively used, such as nanoparticle-based diagnostic assay (10). The continuous monitoring of test accuracy and reliability in the Medical Commission Laboratory is required in attempting to improve the performance of the laboratory and enhance the laboratory quality management system.

CHAPTER 2: STUDY OBJECTIVES

The continuous monitoring of test accuracy and reliability in the Medical Commission Laboratory is required in attempting to improve the performance of the laboratory and enhance the laboratory quality management system. This study aims at determining the reliability of applied tests and identify the gaps in knowing the differences between the different techniques. If discrepancies are indicated, we will utilize acquired knowledge to improve protocols and eliminate inefficiencies to make the testing processes more consistent (reliable), repeatable, and reproducible. The ultimate goal of the study is to limit the spread of communicable diseases in the community, by accurately detecting the infections in newcomers. Besides, this study considered the first of its kind to use large sample size and 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

Although plague jaundice has existed since old civilization, the discovery of viruses as the main etiology of the disease was not known except in the last few decades (1). In 1989, HCV was discovered as the main cause of hepatitis (non-A and non-B). More liver illnesses were also found to be correlated to HCV, with the virus being the main cause of persistent liver diseases around the globe.

3.1. Global burden of the disease

The number of HBV and HCV cases are in a continuous increase (1). The most updated report from the World Health Organization (WHO) had shown that the number of people infected with HCV is around 185 million. On the other hand, 240 million people were reported to be infected with HBV and are always subjected to develop cirrhosis and hepatocellular carcinoma (HCC), which in turn might lead to their death. Every year there are about 780,000 people who are newly infected with HBV (11). Moreover, the number of mortality rate recorded due to HBV and HCV can reach up to 1.3 million individuals annually around the world, where one in every three infected individual dies from the infection (1). Such fatalities are due to severe illness, chronic infection, cirrhosis, and HCC. Twenty percent of the disease arising from HCV Infection occurs in Africa specifically in the Sub-Saharan Area (SSA). In Africa, the numbers of HCV infections seem to be higher than those recorded in Europe and the USA, however, there is an essential need to have more accurate information and investigations on those numbers due to the lack of available reliable diagnosis in SSA (12).

3.2. Types of hepatitis diseases

There are five well-known viruses that cause nearly all hepatitis infections (1).

1. Hepatitis A virus (HAV) causing HAV infection.

2. Hepatitis B virus (HBV) causing HBV infection.

3. Hepatitis C virus (HCV) causing HCV infection.

4. Hepatitis D virus (HDV) causing HDV infection.

5. Hepatitis E virus (HEV) causing HEV infection.

3.3. HCV

3.3.1. Prevalence (Geographical Distribution) of HCV worldwide

It is estimated that three to four million people are infected every year, and 350,000 individuals die yearly from HCV related causes (13). An evaluated 71 million individuals in the world are living with HCV, many of which stay asymptomatic at the early stages; however, they still require treatment (12). Figure 1 shows the global distribution of hepatitis C (1).

Hepatitis C prevalence (percent of population)
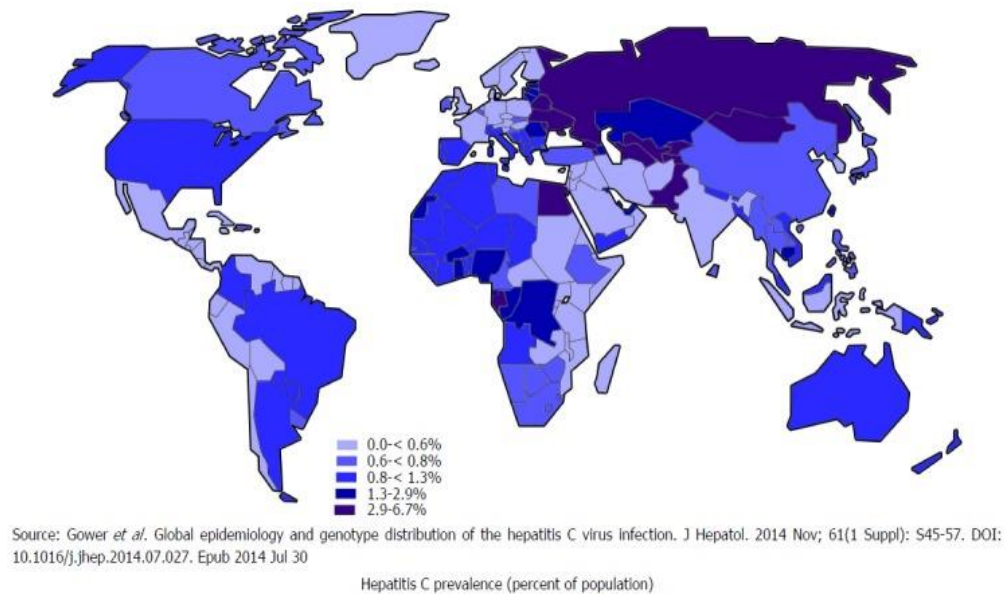
Figure 1: Prevalence of HCV worldwide

3.3.2. Prevalence (Geographical Distribution) of HCV regional

African and Asian nations have the most HCV predominance, while industrialized nations in America, Europe, and Australia have low predominance (14). In the MENA region, Egypt reports the highest predominance of HCV infection, reaching 22% (3). According to WHO, the number of HCV infections was reported with a prevalence of 71 Million worldwide in 2017. The incidence was estimated as 1.75 million new HCV infections per year. It was reported to be 15% in the Eastern Mediterranean Region (15).

3.3.3. Prevalence (Geographical Distribution) of HCV Qatar

Qatar is characterized by a low prevalence of HCV, with a rate of 0.82% in the total population, and only 0.2% among Qataris (2013-2016 estimates). Tackling the issue of HCV in Qatar is not an easy task due to the rapid growth of the community with the convergence of immigrants from hepatitis high-burden countries (5).

3.3.4. Morphology and classification

In 2014, HCV was classified into seven significant genotypes and 67 sub-categories. Several additional subtypes have been established worldwide, which are 1a, 1b, 2a, 2b, 2c, 3a, 4a, 4d, 5a, and 6a. Subtypes typically differ by >15% in their genome sequence. Thus, HCV includes a high degree of heterogeneity through the genome (5). HCV belongs to the *Flaviviridae* family that has a single-stranded RNA genome, with icosahedral symmetry. Its viral spikes on the outer layer of the virion are nearly 6 nm in size, and they shape heterodimers of E1 And E2 Glycoproteins (3).

3.3.5. The entry of the virus

The virus binds to hepatocytes at first, as its host cells. Usually contributed to an interaction between HCV E1–E2 envelope Glycoproteins and Glycosaminoglycans (Chokes) as seen in (Figure 2) (3). Due to an affiliation of HCV with (exceptionally) Low-Density-Lipoproteins [(V)LDL], the LDL receptor (LDLr) has also been proposed as a capture molecule. (16), Figure 3 explains the virus entry (17).

3.3.6. Translation and replication

Viral RNA discharged into the cytoplasm is deciphered through an inner ribosome section location (IRES) found within the 5′ non-translated locale of the genome (Board 2). The RNA encodes a ~3000 amino acid long polypeptide that is split into ten distinctive items. Processing is done by host cell signal peptidases and two viral enzymes, which are cysteine protease dwelling in nonstructural protein 2 (NS2) and (NS3) serine protease that is actuated by communication with NS4A(16). Modes of Transmission It is known that HCV is transmitted through three different ways (2):

1. Blood Transmission

2. Vertical Transmission

3. Sexual Transmission
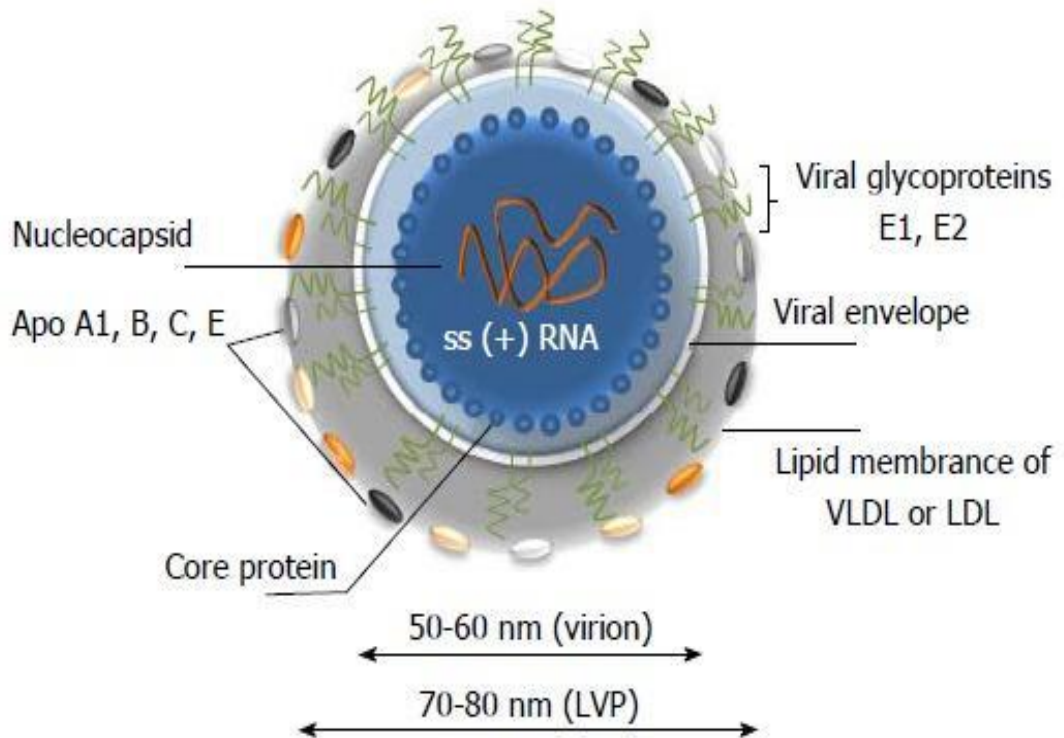
Figure 2: shows the model of the HVC lipoviral particle.

The most common route of the transmission globally was reported to be the blood transfusion, especially in the period before the start of blood screening in 1990. Furthermore, Organ transplantation, and the reuse of syringes, medical instruments, catheters had also been shown to be a common source of infection. Moreover, HCV infection had been reported in cases that are subjected to endoscopy and hemodialysis (3).
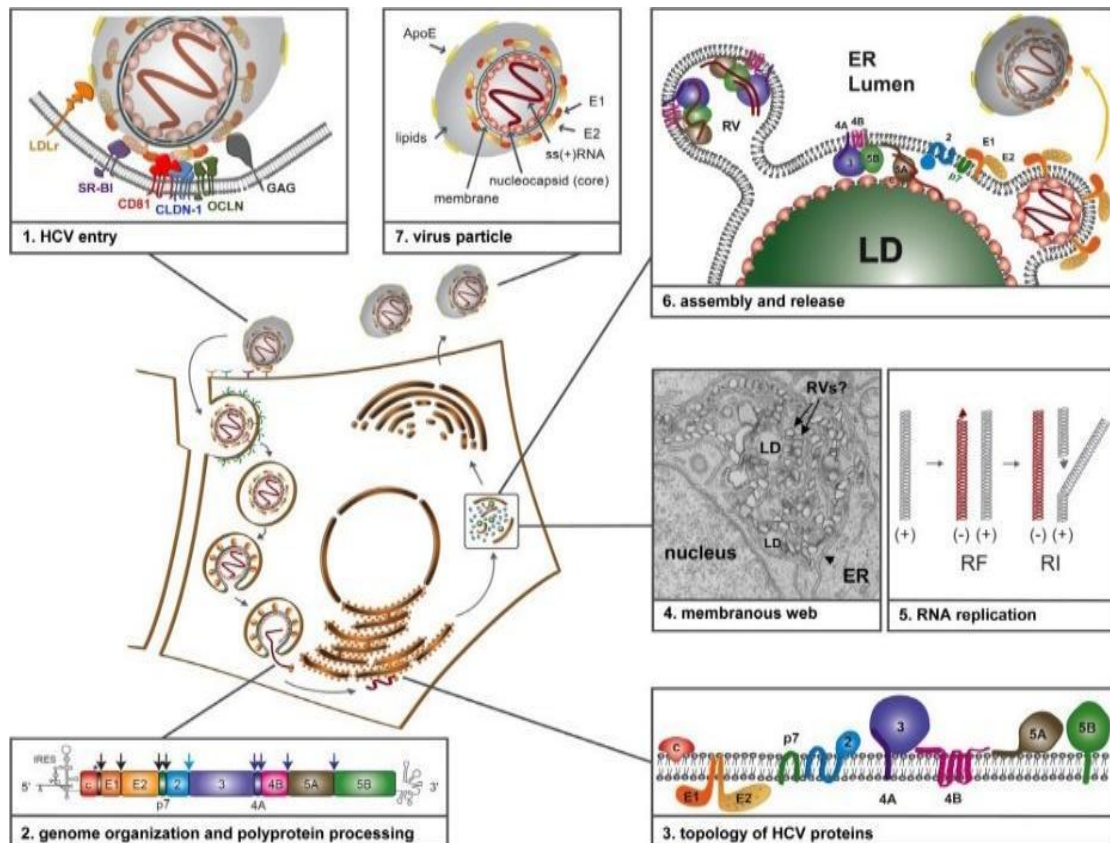
Figure 3: Replication cycle of HCV

3.3.7. Pathogenesis

A. Factors affecting HCV infection

a. Host factors

Host factors in genes related to the immune response may affect the course and outcome of HCV. Among those factors are different types of cytokines, chemokine, and interleukins that combat the virus on different levels during the infection. Furthermore, natural killer cells and T cells such as cytotoxic lymphocyte-associated Antigen 4 (CTLA4) have also been shown to participate in limiting the spread of the virus within the body (18).

b. Viral factors

HCV diversity is the main viral factor that plays a role in disease development, control, and reaction to viral therapy. This is because it has previously been reported that the response to interferon therapy differs according to the HCV genotype. Specifically, genotype 1 and 4 infected patients showed poor response to the interferon therapy alone when compared with those infected with genotypes 2 or 3.

However, the genotype 1 infected patients responded well to the combination of interferon and ribavirin treatment, where they reached Sustained Virologic Response (SVR) of 40–50%. In contrast, patients infected with genotypes 2 and 3 showed an SVR amount of 75%. Besides this significant effect of different genotypes, differences in the HCV genome and the virus load starting point, are considered two additional factors that play a role in the response against the virus treatment (18). Although this dramatic effect has been seen with different genotypes, the effect on HCV infection is minor in the long term, where all genotypes have been reported to show severe liver diseases. However, patients infected with genotype three have shown to be associated with a high possibility to develop liver steatosis(4).

B. The lack of protective immunity

It is believed that there is a role for adaptive immunity in virus protection. This important immune component was concluded from the fact that twenty-five percent of infected patients cleared the virus without treatment. The majority of those patients (83%) were capable of overcoming any recurring virus infection. This was seen from the low virus load detected in those patients as well as their rapid ability of virus clearance(19).

C. Risk factors for reinfection

Several individuals are considered to be at high risk of infection with HCV which include drug users, males having sex with males, illegal sexual activities, and HIV infected patients (13). All those individuals are at high risk of re-infection with the virus; therefore, these individuals are subjected to mandatory screening for the virus before and after treatments. Screening could be done by antibody detection i.e. an elevated level of antibodies against HCV (19).

D. Genetic susceptibility

Several specific alleles had been reported to be strongly associated with HCV occurrence and clearance. Of these, DRB1*1101-DQA1*0501- DRB1*0401- DQA1*03-DQB1*0301 haplotypes had been reported with the virus clearance and/or infection(20). For example, DQB1*0301 showed a crucial role in the self-limitation of HCV infection in different populations. Moreover, the IFNk4 gene was found to be correlated with the clearance of the virus in several cases. Such clearance depends on targeting specific and multiple virus epitopes by T cell responses (CD4+ and CD8+) or by the interferon ISG dependent expression in hepatocytes (20, 21).

3.3.8. Diagnosis

A. Serological methods

Serological measures are frequently utilized as the essential line of a testing method for assurance of ingrained infection with HCV Disease, to rule in those who might be potentially infected and so advantage from the evaluation for treatment. Given that no one test is ever 100% sensitive or specific, a couple of uninfected individuals may likely be ruled-in as HCV; while several infected individuals may be ruled-out from HCV. Both Anti-HCV may be tested utilizing serum or plasma examples with laboratory-based protein Enzyme Immunoassays (EIAs).

All testing methods should aim at the accuracy of the test so it is as close as possible to 100% to minimize misdiagnosis chances. If the test determines antibodies to HCV in an individual, then the individual has already been subjected to HCV. Having said that, only 60%–80% of those individuals can be infected and HCV viremia, while the infection is likely to be cleared in the rest (7).

Testing for antibody responses against hepatitis C infection (anti-HCV) is suggested for the initial identification of the contaminations. An S/CO is a laboratory comparison of some measurable feature of a specimen to the standard set by the laboratory's positive control. Samples with an S/Co ratio of ≥1.0 are defined as positive. A signal-to-cut-off value (S/CO) can be used so that no other tests are needed to be conducted(1). Results that fall below S/co >3.0can be found in non-infected patients and are considered to be false positive (1). Additionally, the utilization of quantitative PCR tests for HCV testing is essential for the appraisal of unremitting hepatitis ailment (7). The Anti-HCV test was created, to begin with, by enzyme-linked immunosorbent test which includes a great affectability and specificity.It has been replaced by automated chemiluminescent immunoassay (CLIA) since the research facility computerization patterns and preferences that had enhanced affectability and specificity.

In any case, in certain clinical settings, the chance of false-positive anti-HCV result is uncommon. This is because the larger part of people undergoing tests have proven of liver disease and the affectability and specificity of the screening measures are high. However, among the population with a low (<10%) prevalence of HCV infection, false-positive results do occur. On the other hand, false-negative results do occur, which is particularly problematic in asymptomatic peoples with no clinical data available, or in those who are being tested for the first time.

Since the plausibility of false-positive results happens, especially in low-prevalence settings such as testing of blood benefactors. On the other hand, positive anti-HCV EIAs are usually confirmed with extra tests such as Recombinant Immunoblot Measures (RIBA) and strip immunoblot measures (SIA such as INNO LIA).

These tests utilize the same proteins as EIA tests of the same period, but the antigens are customarily autonomously localized to choose the number and character of antigens to which anti-HCV antibodies are facilitated. Further, the clinical practice after distinguishing a positive Anti-HCV EIA test is the degree of HCV RNA to assess whether viremia is displayed (22). The fourth-generation anti-HCV Enzyme-linked Immunosorbent assay (ELISA) detects HCV capsid antigen and antibodies to the core, NS3, NS4, and NS5 regions of the virus(23). The HCV core antigen test can be utilized in resource-limited settings. It is a profoundly precise immunoassay that can decrease the high rates of checking. HCVcAg measures may, moreover, be valuable as a one-step screening test in a high predominance population.

Any case could be a two-step Chemiluminescent Microparticle Immunoassay (CMIA) (Modeler HCV Ag), which appeared high affectability and specificity of HCVcAg (24). The serologic demonstrative tests begin by utilizing the steps for recognizing the infection, without distinguishing between acute and chronic infection. Examinations for patients with HCV disease incorporate serological measures for antibodies to Hepatitis C (hostile to- HCV) and atomic tests for the location of Viral RNA (8).

B. Molecular methods

One of the major problems in the diagnosis using serological methods is the lack of detection of the active virus during the early phases of infection or viremia before the formation of detectable levels of antibodies(8). The molecular diagnostic methods appear to resolve this problem with higher specificity and more sensitivity as compared to the serological ones. Because of their importance in diagnosis, those assays become currently available at affordable and economical prices that can accommodate the economy of poor nations that have a high prevalence of the virus. One of those methods is the Nucleic Acid Test (NAT), which is considered to be the most advanced, more reliable, highly accurate, very specific, and sensitive among all techniques(8).

NAT is used as a principal test to detect the genome of different viruses in the sample. Also, it can be used as a confirmatory test for the positive serological test. Hence, this is a test that can be applied for the diagnosis of HCV in patient samples as primary detection as well as confirmation for the presence of the viral RNA. The NAT procedure is standard among all known methods that start with viral nucleic acid purification and followed by its amplification in a thermal cycler.

A crucial point in this method is the correct choice of the primers that specifically bind to the conserved regions present in the virus genome. In the case of Viral RNAs like HCV, a step of reverse transcription is added to generate cDNA for PCR amplification. One of the known NAT available is the Cobas Taq Screen MPX test that has been used in the detection of different viral genomes such as HCV (RNA) and HBV (DNA) (25). NAT could be qualitative or quantitative, the qualitative method is known as conventional PCR or RT-PCR (Real-Time PCR), where the results are either showing that samples are reactive or non-reactive, whereas the Quantitative method accurately reflects the amount of the virus genome. Quantitative (q) RT-PCR detects the expression level of that fragment in the organism. And is known for its high

specificity and sensitivity that reaches 99%(23). Indeed, Albertoni et al. (2014) were able to detect HCV RNA as low as 310 IU/ml using qRT-PCR(25).

Molecular methods used to detect HCV ranges from subjective HCV test to deep analysis of the genome. A subjective test is used to identify circulating HCV in the blood, which is considered an important initial step in HCV diagnosis that screen for infected persons. Moreover, the detection of viral RNA is crucial for the diagnosis of infected individuals, carriers, seroconverted cases, and to differentiate active from dormant cases.

The advantage of molecular methods is not limited to screening only but also extends to genotyping. This method helps to distinguish between different genotypes as there are about seven virus genotypes and more than eighty HCV subtypes. Besides NAT, hybridization and sequencing methods could be used for a more deep analysis of the virus genotype(8).

In case NAT is not available, because of the limitation in resources, other economically affordable methods, ELISA and CLA, could be used to detect HCV core antigens. Importantly, it has been found that the detection of HCV core antigen is a very effective diagnostic method as compared to NAT. Based on this, the new Architect HCV Ag Test was developed by Abbot company to be one of the sensitive methods to detect HCV in patient samples (23).

3.4   HBV

3.4.1   Prevalence (Geographical Distribution) HBV Worldwide

Depending on the WHO report, around two billion individuals are infected with HBV around the world, and about 600,000 people die annually due to its acute or chronic consequences. In other words, roughly one-third of the World's population has been affected by HBV(1). (Figure 4) shows the prevalence of HBV worldwide (1).
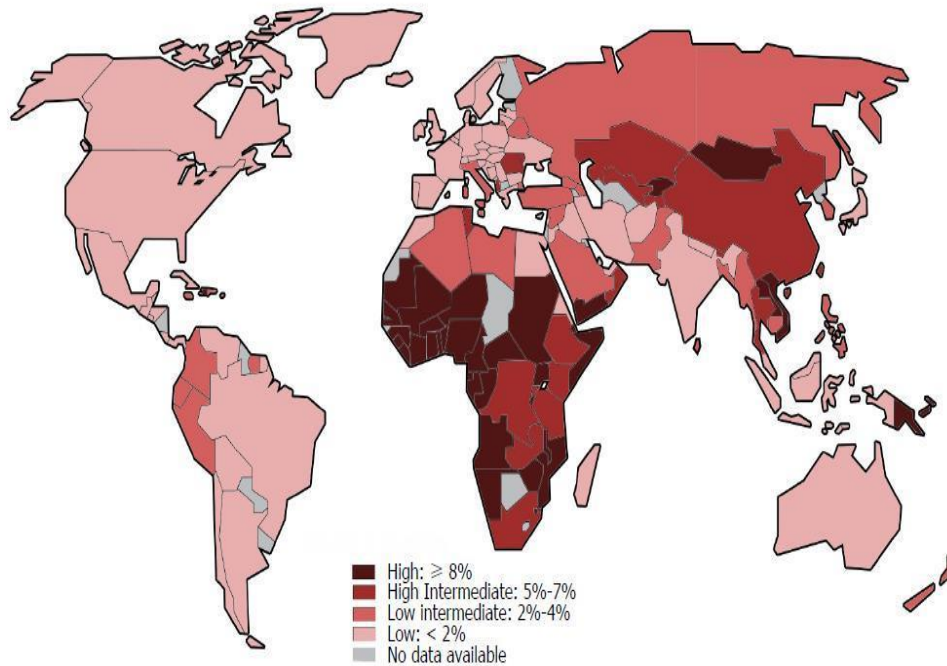
Figure 4: Figure 4 Prevalence of HBV worldwide.

3.4.2 Prevalence (Geographical Distribution) HBV regional

Among the globe, SSA is considered to have a high level of HBV infection within its population, where about 6.1% of Africans caught the virus and developed the disease. Similarly, in the southern and eastern parts of Asia, HBV infection was reported in about 100 million cases. On the other part of the globe, in the Caribbean and Latin American countries, the percentage of HBV infected individuals reached ~1% of the population. Those countries had also shown a high number of HCV infected patients (7-9 million) in association with HBV. Moreover, in the Middle East region, HBV cases were reported in about 3.3% of the population. In contrast to those regions in the world, North America showed the lowest HBV prevalence(1). Regarding WHO 2015, Western Pacific regions accounted for 68% of all infected persons.

3.4.3 Prevalence (Geographical Distribution) HBV Qatar

In Qatar, the prevalence of HBV is less than 2%, putting into consideration that the population in Qatar is a very heterogeneous population, where the highest percentage of the individuals are expatriates coming from different parts of the globe. The highest numbers are from Asia that is being recorded as one of the highest numbers of HBV infections(5).

3.4.4 Morphology and classification

HBV may be a part of the Hepadnaviridae family where HBV genotypes contrast by more than 8%. Additionally, eight genotypes of HBV, called A, B, C, D, E, F, G, and H, can be recognized whereby, these sub genotypes vary by at least 4%. In genotypes A, B, and C, epidemiological information appears that the individual sub genotype sets A1/A2 (once named Aa/Ae), B1/B2 (once in the past Bj/Ba) and C1/C2 (once Cs/Ce) contrast significantly in numerous virologic and likely a few clinical parameters. All HBV genotypes can be isolated into sub genotypes but E and G. (Figure 5) shows the genotypes of HBV (26).
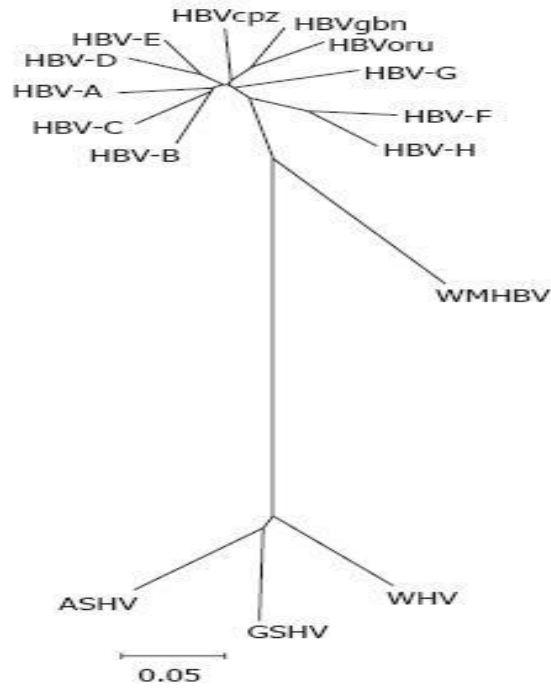
Figure 5: Appears the genotypes of HBV
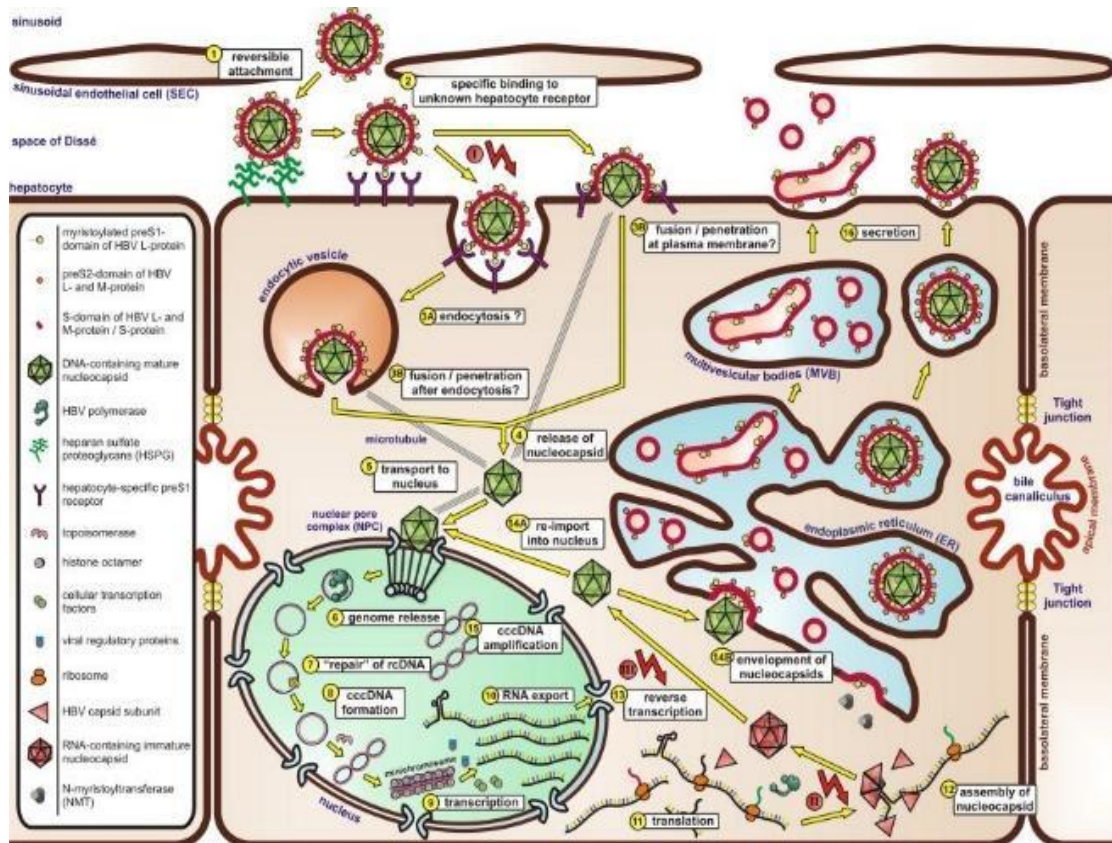
Figure 6: Replication cycle of HBV

3.4.5 The entry of the virus

The entry begins with reversible and non-cell-type explicit relationship to cell-associated heparan sulfate proteoglycans, then specific and possibly irreparable binding to associate unknown hepatocyte-specific preS1-receptor. This step presumptively needs activation of the virus, thus leading to the presentation of the myristoylated N-terminus of the L-protein. In this case, two diverse section pathways are proposed: endocytosis taken after by unharness of nucleocapsids from endocytic vesicles; and a combination of the infective operator envelope at the cell divider (27).

3.4.6 Translation and replication

One of the important steps in the virus life cycle is the replication phase. This starts with the presence of the viral nucleocapsid in the nucleus, which is followed by the delivery of the rcDNA into the nucleoplasm. Then this is followed by a ligation process of the two DNA strands which is considered a crucial step for the formation of cccDNA. This special piece of DNA consists of non-histone as well as histone proteins and it is a critical molecule synthesized by the virus to use the cell on different levels of transcription and translation to synthesize the Viral RNAs and produce viral proteins that are important for both replication and assembly (27). Figure 6 shows the Replication cycle of HBV (28).

3.4.7 Modes of Transmission

HBV transition routes include percutaneous (i.e., puncture through the skin) or mucosal (i.e., direct contact with mucous membranes) exposure to infectious blood or body fluids(29) It is transmitted by parenteral route, sexual and vertical transmission (2).

3.4.8 Pathogenesis

A. Factors affecting HBV infection

a. Host factors

Several host elements had been detailed to be included in virus replication. Some of these factors help in transcription, whereas others play a role in translation. For example, the CCAAT/enhancer-binding protein (C/EBP) and hepatocyte nuclear factors (HNF) are cellular transcription factors that mediate transcription as well as viral gene expression. The virus recruits those proteins to interact with the promoters display in its open perusing outlines (ORFs) (four overlapping ones) to produce different virus structural and non-structural proteins, like the nucleocapsid, precure protein, the envelope proteins (M, L, and S)(27).

b. Viral factors

Viral proteins (center, the administrative X-protein) control the method of translation and may change viral quality expression by collaboration with the viral promoters of the four major (ORFs). Viral variables can control the cccDNA arrangement. As HBV polymerase inhibitors do not specifically influence the cccDNA, a diminish in cccDNA levels is assumed to infer from the need of adequate reusing of viral nucleocapsids to the core, due to the restraint of viral DNA-synthesis within the cytoplasm, and less approaching infections from the blood(27).

c. Environmental factors

Natural components are involved in HBV pathogenesis. Liquor and aflatoxin were found to influence the movement of persistent Hepatitis B. Liquor utilization increases the seriousness of the liver infection and raises the chances of creating liver decompensation from cirrhosis. Patients with unremitting Hepatitis B presentation to aflatoxins are at an elevated risk of HCC(30, 31). Change of living conditions might modify the immunological reaction, in this way favoring HBeAg immune

clearance(32).

B. The lack of protective immunity

HBV infection could result in either acute (more than 90% of the cases) or chronic illness. HBV infections are among the top ten causes of death worldwide (Hunt,2007). Both antibodies and cytotoxic T cells coordinated to diverse HBV antigens play a vital part in diminishing viral load while clearing HBV-Infected hepatocytes from the liver. Unlike grown-ups who generally do not develop the persistent disease, neonates, and newborn children who acquire HBV disease from their moms at birth, are most vulnerable to the foundation of persistent HBV disease. Having a safe reaction plays a fundamental role in HBV-related hepatocyte destruction since the virus itself is not cytolytic (11).

C. Risk factors for reinfection

HBV reactivations and reinfections are usually associated with immunosuppression, such as that found in HIV infection. Also, relatively low Anti-Hbs levels and discontinuation of Truvada (tenofovir disoproxil fumarate and emtricitabine) can cause re-infection (33).

D. Genetic susceptibility

As described by Zhang et al. (2019), HLA-DPA1 rs3077 and HLA-DPB1 rs9277535 are correlated with chronic HBV infection. HLA-DPA1*0202-DPB1*0501 and HLA-DPA1*0202-DPB1*0301 are also associated with susceptibility to chronic hepatitis B whereas HLA-DPA1*0103-DPB1*0402 and HLA-DPA1*0103-DPB1*0401 can have a protective effect. Also, associations between genes encoding cytokines (including IL-1, IL-4, IL-6, IL-10/IL-10RB, IL-12/IL-12B, IL-18, IL-27, IL-28B, IFN-γ, TNF-α, and TGF-β and chronic HBV infection have been reported (6).

3.4.9 Diagnosis

A. Serological methods

The essential conclusion of HBV diseases is made by utilizing serological tests for identifying antigens and antibodies against this infection. Hepatitis B infection (HBV), maybe a somewhat double-stranded DNA infection, a species of the class Orthohepadnavirus, and a part of the Hepadnaviridae family of infections.

HBV infection is typically detected by immunoassays that use HBV to capture antigens in the sample. As with most of the serological assays, nonspecific binding can occur. Manufacturers determine a cutoff value that balances the necessary high sensitivity for detecting the antigen (or Anti-HBsAg antibody) while avoiding false-positive results (2). HBsAg, Anti-HBs, HBeAg, Anti -HBe, Anti -HBc IgM, and IgG are all serological markers that can be used in the diagnosis of HBV. HBsAg and Anti -HBs can be detected in patients with HBsAg positive and they are regarded as carriers of HBV (34).

The Hepatitis B surface antigen (HBsAg) is the main serologic diagnostic tool of HBV infection and is a very sensitive and specific biomarker. Chemiluminescence (CLIA) and electrochemiluminescence technology (ECLIA) are now used for extremely sensitive immunoassays on automated analyzers for HBV serology.

However, since HBsAg assays are highly sensitive, false positives can occur, and therefore, when HBsAg index values are close to the cut off value and are inconsistent with other serological markers, then another confirmatory assay is recommended. Standard enzyme-linked immunoassay (ELISA) can be used for diagnosis as it is not expensive and does not require much instrumentation; it has high sensitivity (>99%) and acceptable specificity (>95%) for HBsAg (35).

HBeAg is used to detect the exact phase of the disease and its seroconversion from positivity to negativity often occurs which is associated with reduced morbidity

and mortality provided HBV DNA is low. HBcrAg or Hepatitis B core-related antigen consists of antibodies that can detect three proteins, HBcAg, HBeAg, and p22cr which is a small core-related protein found in DNA-free empty particles. HBcrAg can be used as a marker for intrahepatic replication, especially in cases with HBeAg seroconversion (36).

The HBsAg ELISA Kit was used for the diagnosis of HBV. As described by the researchers, the HBsAg Antigen covers the titer wells of the plate. Then HBsAb antibodies in serum or plasma are added to the wells together with an HBsAg conjugated with peroxidase. After that Antigen-Antibody–Antigen complex represented by the HBsAg conjugated to peroxidase are formed. HBcAb ELISA Kit microtiter wells are covered with HBV antigens. Specimens containing Anti-HBcAg Antibodies (HBcAb) and Anti-HBcAg (HBcAb) will bind to the limited number of solid-phase sites(37).

Anti-HBc or Hepatitis Core Antibody can detect both past HBV infection and existing HBV infection. Titers of Anti-HBc can predict therapeutic response and also a lack of need for HBV vaccination (Jackson et al.,2018). HBcrAg or Hepatitis B core related antigen can also be used as a marker for HBV. It quantifies HBcAg, HBeAg, and core related protein p22 (p22cr) in one test. Some studies also have postulated that it can be used as a potential biomarker in both HBeAg positive and negative phases of the infection (35).

B. Molecular methods

The development of Hybridization and Polymerase chain reaction (PCR) assays for direct determination of HBV-DNA is a product of advances in molecular biology techniques. The diagnosis of HBV infection can also be made by the detection of HBV or Hepatitis B core Antigen (HBcAg) in liver tissues by immunohistochemical staining

and of HBV DNA by Southern hybridization, in-situ hybridization, or PCR(38). HBV DNA can detect the replication activity of the virus and is considered a reliable marker. This can include Signal Amplification, Target Amplification as well as Real-Time PCR which has come to be the standard method to detect and quantify HBV DNA in clinical settings (34).

HBV DNA can show that actively replicating viruses within the hepatocytes is produced. It also helps in determining the stage of the disease and in monitoring the effect of NA therapy(36). Durgadevi et al. (2012) used a combination of serological and molecular methods to diagnose HBV, through serum and dried blood spots. Complete serological tests were performed and also PCR was performed on serum samples and dried blood spots. They concluded that using dried blood spots in detecting HBV DNA can be used as a convenient method of testing in resource-limited settings, as the results are comparable to serum tests(39).

There are several sensitive real-time PCR assays available for quantification of HBV DNA in plasma or serum. Examples include the Roche COBAS TaqMan HBV Test, Abbott RealTime HBV Assay, Siemens VERSANT HBV DNA, and Qiagen artus HBV RG PCR(35). Kurdi et al. (2014) mentioned the COBAS diagnosis test for HBV DNA quantification in human plasma and serum, which is a nucleic acid amplification test. This test consists of first of specimen preparation to isolate HBV DNA then performing PCR amplification of target DNA and at the same time deception of cleaved dual labeled Oligonucleotide detection probe specific to the target. Their study concluded the superiority of the PCR method over ELISA as it was more sensitive and reliable than the ELISA technique. They recommended the use of PCR methods as an accurate and reliable method for the detection of HBV(37).

3.5 Medical commission

The Medical Commission of the Ministry of Public Health works effectively towards the implementation of a strategy consisting of the importance of functions. The Medical Commission is screen for residents and all travelers to the country who are then tested to ensure that they do not carry diseases and therefore, help the society be limited the spread. In Qatar, the Medical Commission department screened around 607,601 individuals in 2019, with an estimated average of 2470 visitors per day. The department is mainly responsible for screening newcomers and residents for the following purposes:

- Health screening is done for obtaining residence permits as well as medical fitness certificates while applying for a job or continuing university study.

- Annual Health screening is done to obtain a health certificate for people who are working as food handlers, barbers or beauticians, as well as obtaining a QCHP license and changing one's employer.

The Medical Commission is mainly composed of the medical section, radiological section, and laboratory section.

3.5.1 Laboratory section in Medical Commission:

It conducts laboratory examinations daily as routine work by serological diagnosis mentioned below:

3.5.1.1 ARCHITECT Immunoassay:

It is utilized for the qualitative detection of antibodies to presumed structure and non-structure proteins (NS3 and NS4) of the HCV as well as the detection of hepatitis B surface antigen (HBsAg) of HBV in human serum and plasma In addition to detect HIV P24 antigen (Figure 7) (40).

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Architect immunoassay I Abbott types:

1-The ARCHITECT i2000SR immunoassay analyser

- It has 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 7: ARCHITECT Immunoassay

3.5.1.2 INNO-LIA HCV Line Immune Assay (LIA):

The INNO-LIA can be uses to detect HCV and HIV viruses; For HCV score is a Line Immune Assay (LIA) that is used to help detect the presence of antibodies to HCV in human serum or plasma. The goal of this test is to be used as a supplementary test on human serum or plasma specimens (Figure 8) (41). That usually found to be reactive using an anti-HCV screening procedure. Besides, it is also considered as a third-generation line immunoassay which helps incorporates HCV antigens derived from the core region, the E2 hypervariable region (HVR), NS3 helicase region, NS4A, NS4B, and NS5A regions.(Figure 9) (42).

Figure 8: INNO-LIA HCV Line Immune Assay (LIA)
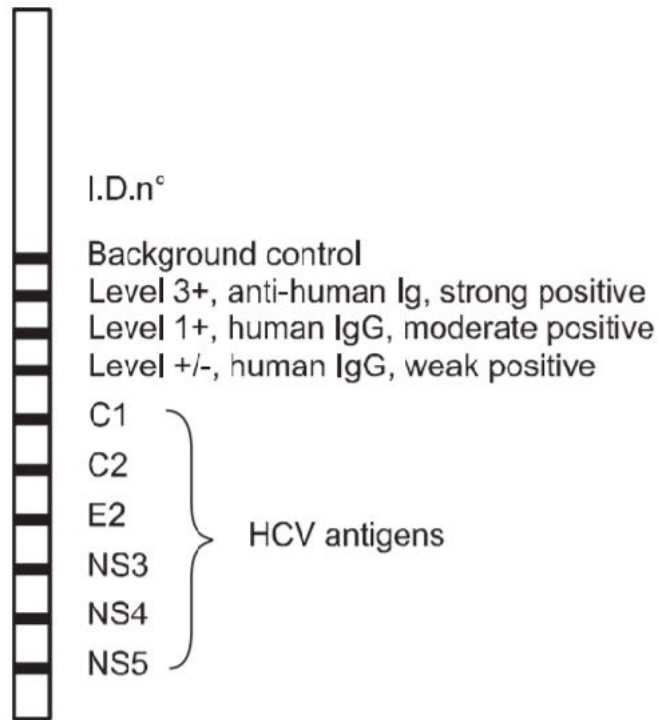
Figure 9: INNO-LIA HCV Score test strip. The intensity of the reaction on the control lines on each strip is used to assign the reactivity ratings for each antigen on that strip. If the intensity of antigen line reaction (R) is: 1) Lower than ±, then the rating is negative 2) Equal to ± then the rating is ± 3) Higher than ±, but lower or equal to 1+ then the rating is 1+ 4) Higher than 1+ but lower than 3+ then the rating is 2+ 5) Equal to 3+ then the rating is 3+ 6) Higher than 3+ then the rating is 4+

3.6 Routine of work

ARCHITECT is a chemiluminescent microparticle immunoassay (CMIA) that is often used for detection several viruses most frequently the ARCHITECT is used for HCV, HBV, and HIV antibody/antigen detection with slight differences in the procedures. For instance, the ARCHITECT Anti-HCV antibody is regarded as a two-step immunoassay, whereas Anti-HBsAg qualitative II is a one-step immunoassay. The latter makes use of the technology of chemiluminescent microparticle immunoassay for the qualitative detection of Anti-HCV and Anti-HBS.

Regarding anti-HCV testing, the sample will be added to a microparticle coated with a recombinant HCV antigen. If the sample has an anti-HCV it will bind to the antigen coated in the microplate. Consequently, creating a reaction mixture when Anti-human IgG/IgM acridinium-labeled conjugate is added after washing. Moreover, the reaction mixture also requires the addition of trigger and pre-trigger after another wash cycle. In this case, Relative light units (RLUs) are usually used in measuring the reaction resulting in chemiluminescent (43). The specimen is considered non-reactive (NR) if cutoff (S/CO) values < 1.00. In addition, reset in duplicate results is where the criterion of ARCHITECT Anti-HCV used when the reactive specimen is considered repeatedly responsive for Anti-HCV in case of one or both results being reactive. However, for Anti-HCV, the sample examined is considered non-reactive if both results are nonreactive (Figure 10). Further investigation is required in repeatedly reactive for Anti-HCV specimens in supplemental tests like INNOLIA assays, other HCV specific immunoassays, or a combination of PCR test. The specificity of ARCHITECT anti-HCV is 99.60% with a 95% confidence interval while the sensitivity is 99.10% with a 95% confidence interval. There is no detection limit of ARCHITECT anti-HCV because the test is a semi-quantitative test using a signal of a cut off value.

For HBsAg qualitative II testing, similar procudeure as in anti-HCV will be followed except that the microplate is coated with anti-HBs. The creation of a reaction mixture requires the sample of Anti-HBs acridinium -labeled conjugate and Anti-HBs paramagnetic microparticles (44). The specimen which is considered Retest in duplicate (Reactive) are those with S/CO values <OR = 1.00. In Duplicate Reset Results for HBsAg, the specimens are considered nonreactive if both results were nonreactive. The specimen is considered repeatedly reactive if both are reactive (Figure 11). This can then be confirmed by a neutralizing assay if reactive. The specificity HBsAg qualitative Confirmatory II is > 99.5% with a 95% confidence interval while the sensitivity is 99.09%, with a 95% confidence interval. In terms of Detection limit, HBsAg assay commercially available was 0.021 IU/ML as per package while adding the upper limit of the 95% confidential interval. For the analytical sensitivity results, linear regression ranging from 0.017 to 0.022 IU/ML was used in calculating the ARCHITECT HBsAg Qualitative II assay.

3.7 Description neutralization test.

A Chemiluminescent Microparticle Immunoassay (CMIA) is the HBsAg Qualitative 11 confirmatory assay in human plasma and serum for the presence of Hepatitis B surface antigen confirmation (45).

3.7.1 Biological Principles of the Procedure

Both one-step pretreatment immunoassay in two single tests is involved in the ARCHITECT HBsAg Confirmatory V.1 assay utilizing Chemiluminescent Microparticle Immunoassay (CMIA) technology with Chemiflex. In a reaction vessel (RV), Pretreatment 1 and sample are combined and incubated. In Pre-treatment 1, the antibody is used to neutralize HBsAg. Creating a reaction mixture requires the combination of Anti-HBs coated conjugate and paramagnetic microparticles and the pre-treated sample aliquot. The Anti-HBs coated microparticles bind with non-

neutralized HBsAg in the sample. Thus, anti-HBs coated microparticles binding with the neutralized HBsAg, is blocked.

The second steps require the addition of acridinium labeled Anti-HBs conjugate. The reaction mixture also involves the addition of trigger and pre-trigger solutions following another wash cycle. Relative light units (RLUs) are used in measuring the reaction resulting in chemiluminescent. The RLUs detected, and the ARCHITECT, which identifies the amount of HBsAg system optics, has a direct link.

3.7.2 Interpretation results

Using the ratio of the samples Relative light unit (RLU), the HBsAg quantitative II Confirmatory assay cutoff (S/CO) results are calculated by the ARCHITECT I system to the cutoff RLU (S/CO). The ARCHITECT I system calculates the % nutralization results for HbsAg quantitative II Confirmatory assay using the HBSAgQ2 and HBSAgQ2 C2 results.

- In all cases of dilution If % Neutralization is not applicable, the final interpretation is not confirmed.

- In all cases of dilution, If % Neutralization > or = 50%, the final interpretation is confirmed positive.

- In case of neat dilution, If % Neutralization < 50%, the final interpretation is Repeat test used in a 1:500 dilution.

- In case of 1:500 dilution If % Neutralization < 50 %, the final interpretation is Go to 1:20000 dilution.

- In case of 1:2000 dilution If % Neutralization < 50 %, the final interpretation is not confirmed.

3.8 Description of HCV quantitative test (The COBAS AmpliPrep/COBAS) Taqman HCV Quantitative Test, version 2.0)

PCR was used for detection HCV and HIV viruses, in our cases these tests offer the ability to qualitatively detect hepatitis C virus or quantitatively determine amounts of HCV circulating in a patient's blood. The diagnosis of acute and chronic HCV infection is based on the detection of HCV RNA by a sensitive molecular method. The qualitative test confirms HCV antibody-positive specimens and provides evidence of an active infection. The COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 is a qualitative in vitro nucleic acid amplification test for the detection of Hepatitis C Virus (HCV) RNA genotypes 1 to 6 in human EDTA plasma or serum. It utilizes automated specimen preparation by a generic silica-based capture technique. It is based on three major processes: (1) specimen preparation to isolate HCV RNA; (2) reverse transcription of the target RNA to generate complementary DNA (cDNA) and (3) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide detection probes specific to the target.

3.9 Description of INNOLIA Biological Principles of the procedure

INNOLIA is used to detect different viruses such as HCV and HIV. For HCV, the antigens are pre-coated on nylon strip and a sample is then incubated in a trough along with the test strip. HCV antibodies, if present, will bind to the HCV antigen lines on the strip. Thereafter, it is proceeded by addition of conjugate to react with specific HCV antigen/antibody complexes, if previously produced. Afterward, incubation with the enzyme substrate forms a chesnut-like color, whose intensity is proportionate to the amount of HCV-specific antibody taken from the sample on any given line. Color development is then stopped with sulfuric acid (42).

3.9.1 Interpretation of the results:

A sample is negative for HCV antibodies in the following two cases:

- If all HCV antigen lines experience a negative reactivity rating.

- If only one HCV antigen line has a reactivity of ±, except when the reactivity is observed for NS3.

On the other hand, a sample is positive for HCV antibodies if at least two HCV antigen lines have a reactivity of ± minimum or higher. Besides, a sample is considered indeterminate for HCV antibodies if one HCV antigen line has a reactivity rating of 1+ or higher and if the NS3 line reacts with a reactivity of ± or higher and all other antigen lines are negative.

Figure 10: HCV Architect Chart

- ✓ If ARCHITECT Anti-HCV Round 2 is positive, INNO-LIA HCV is positive, and PCR is positive → HCV result is confirmed positive
- ✓ If ARCHITECT Anti-HCV Round 2 is positive, INNO-LIA HCV is negative, and PCR is negative → HCV result is confirmed negative
- ✓ If ARCHITECT Anti-HCV Round 2 is positive, INNO-LIA HCV is negative, and PCR is positive → HCV result is confirmed positive
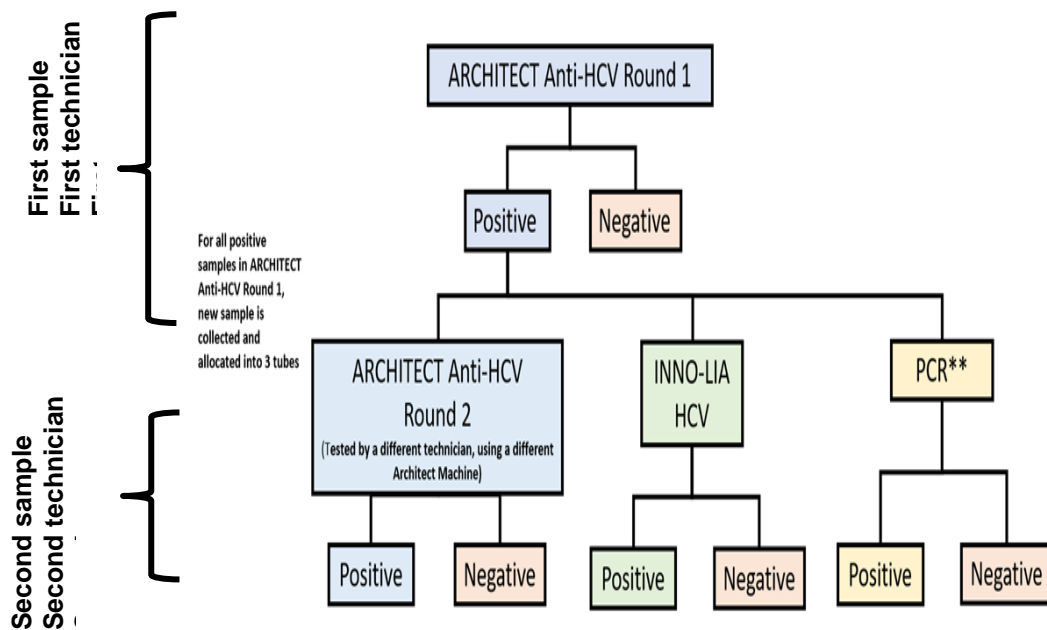- ✓ If ARCHITECT Anti-HCV Round 2 is positive, INNO-LIA HCV is positive, and PCR is negative → HCV result is confirmed indetermined; → Repeat after 4 weeks ARCHITECT Anti-HCV Round 3, INNO-LIA HCV, and PCR
- ✓ If PCR is negative → HCV result is confirmed negative
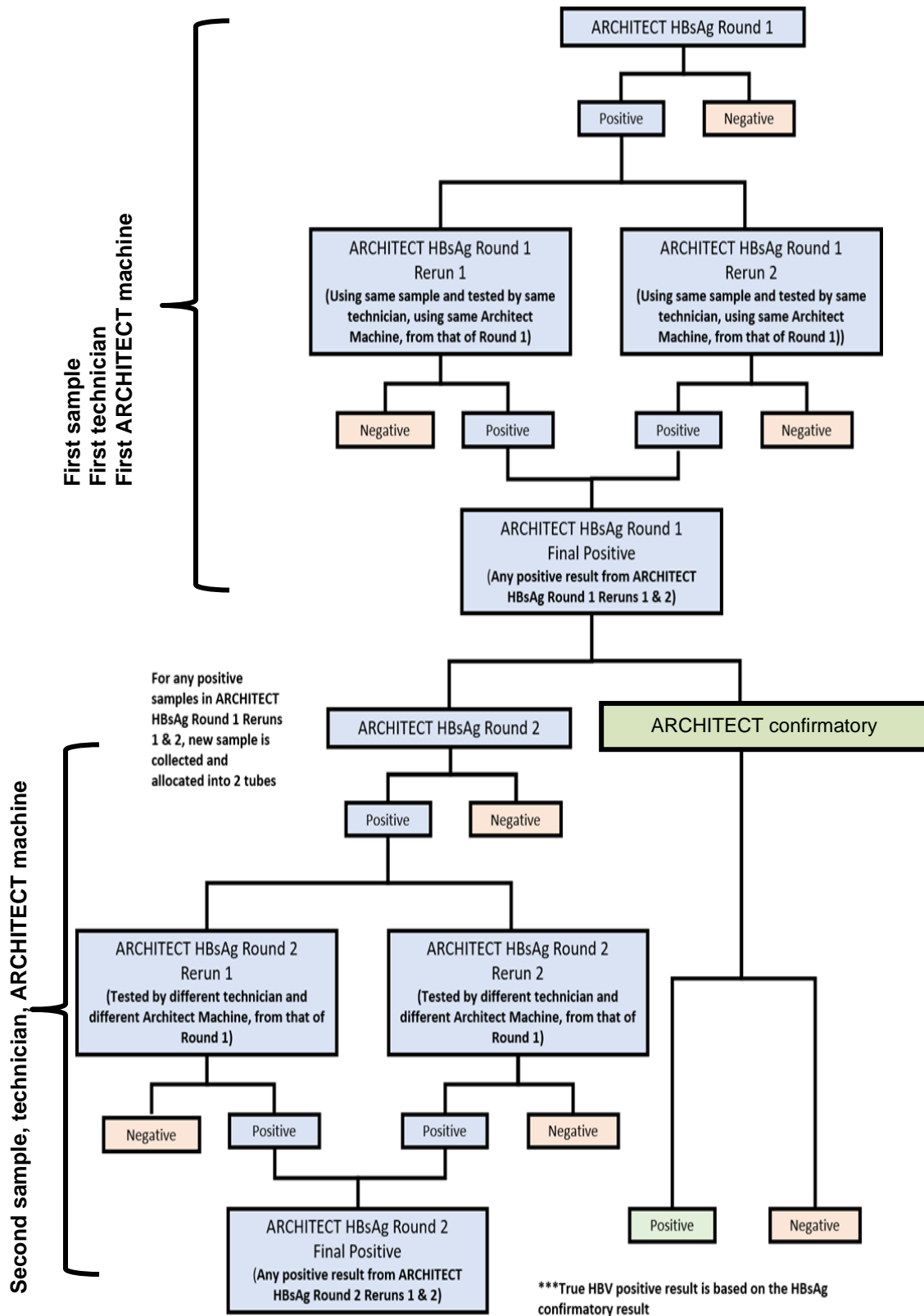
Figure 11: HBV Architect Chart

CHAPTER 4: METHODOLOGY

4.1. Ethical considerations This study granted IRB exemption by the ethics committee of MOPH IRB and Qatar University before the study data collection.

4.2. Inclusion and Exclusion Criteria and number of samples

All initial reactive/positive for Anti-HCV and HBsAg blood samples that were collected between January 1st, 2019, and December 31st, 2019 were included and analyzed.

4.3. Recruitment Methods

No patients/applicants were involved or in other words, there was no direct or indirect interaction with human subjects. The study will be conducted on existing de-identified testing results in the MC database

4.4. Confidentiality

The study was conducted on de-identified data. The results of testing as figures and only data that is non-identifiable will be published. The data was saved in the medical commission's password-protected database medic system, and the students had limited access from the section head for de-identified test results information. All samples were barcoded, and all sample results were stored in the medic system database which was password-protected to protect applicants' identities.

4.5. Data collection and statistical analysis

This Retrospective study was conducted on 1120 hepatitis B (HBsAg) lab results from January 2019 to December 2019, which were tested positive for hepatitis B (HBsAg) using an Architect from Abbott (USA), and thus, included in this study. The replicate ARCHITECT reading was analyzed and was used for repeatability and reproducibility purposes.

4.6. Confirmatory results test

The HBsAg confirmed test results by HBsAg neutralization test of these 1120 samples were also included and analyzed for Positive Predictive Value (PPV), true positive and false-positive results. For HCV testing, 1399 Anti-HCV positive samples during the period of January 2019 to December 2019 were included in this study. The replicate ELISA reading was analyzed and used for repeatability and reproducibility purposes. Anti-HCV confirmed results by HCV INNO LIA, and HCV PCR was also included and analyzed for PPV, true positive, and false-positive results. The date was extracted from the Medic system (Health Information System "HIS"/ Laboratory Information System "LIS") database using an SQL (i.e., Structured Query Language) script in a tool used to execute SQL scripts namely called Toad. Then the output of the SQL script was saved in a CSV (i.e., Comma Separated Values) text file, and then the CSV text file was opened in Excel and was saved as an Excel file. Then we converted the data into the coding system.

4.7. Sample size calculations

The sample size was calculated based on the confidence level using the Stephen Thompson equation (equation 1), which is one of the best equations for calculating the sample size for a study.

Equation 1: Sample size equation

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

- 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
  The data (HCV) was collected from the Medic system program and was saved

on an Excel file. The data was a horizontal form placed in the form of rows so that each row represents a case of the reviewers. Then an excel sheet was used, and the data were coded and classified based on the person reading. If it was greater than or equal to one, then the result was considered positive, and if it was less than one, then it was considered as negative. =IF(D12>=1;"POS";"NEG") and coding POS = 1, NEG= 0.

4.8 Calculation of the different diagnostic measurements

Equation 2: Calculation of the different diagnostic measurements (OPA, PPA, NPA, and PPV).

		Reference Standard		
		Positive	Negative	Total
Assay/Test	Positive	A	B	A+B
	Negative	C	D	C+D
	Total	A+C	B+D	A+B+C+D

- Overall Percent Agreement= (A+D)/(A+B+C+D)

- Positive Percent Agreement = A/(A+C)

- Negative Percent Agreement = D/(B+D)

- Positive Predictive Values =A/(A+B)

CHAPTER 5: RESULTS

5.1 Evaluation of the performance agreement in different HCV diagnostic methods

We analyzed data collected from a total of 1399 individuals who were previously screened for HCV during their application for Qatari residency in Doha in the period from January 1, 2019, to December 31, 2019. The screening process was previously done at the Qatar Medical Health Commission laboratory using the ARCHITECT immunoassay on two rounds using two different analyzers ie. analyzer A (round 1) and analyzer B (round 2). It is important to note that round 2 was performed by another technician to ensure reproducibility. The results obtained from those two rounds were further confirmed by NNO-LIA HCV and PCR analyses (PCR was done at Hamad Medical Corporation).

Our data analysis showed that all 1399 samples screened in the ARCHITECT analyzer A (round 1) were HCV positive. However, in round 2 the number of samples analyzed was only 1393 because this round was based on recollecting blood samples from all positive cases. During this process, six subjects did not come back for the blood withdrawal. In this case, according to the protocol, text messages are usually sent 3 times. If the applicant still does not respond, a verification is usually carried out inside the Ministry of Interior system to ensure that the individual is inside or outside the country. If the individual is outside the country, the file is closed. As for whether he/she is inside the country, a communication with the responsible authorities is carried out within the country to inform them that the person is unfit because the preventative checks are incomplete. Therefore, those six samples were excluded from all our subsequent analyses. Consistent with the results obtained from round 1 screening using ARCHITECT analyzer A, all 1393 recollected samples analyzed in round 2 of the screening were HCV positive (Table 1). Therefore, no sample tested negative and hence

zero value was placed as shown in the table.

Indeed a 100% agreement (overall and positive percent agreements; 95% CI, 99.72-100%) between the results obtained from the two screening rounds was observed (Table 5). To further confirm these results, we analyzed the data obtained from the two other confirmatory diagnostic tests, INNO-LIA HCV and PCR. Using the INNO-LIA assay, we were able to identify 311 as HCV positive, 787 HCV negative and 295 indeterminate (HCV-specific IgG antibodies may or may not be present) (Table 2). We then used these results to investigate the overall percent agreement (95% CI) with the results obtained in round 2 using ARCHITECT analyzer B. Surprisingly, the overall percent agreement was 28.32% (25.73%-31.065), whereas the positive percent agreement (95% CI; 98.78%-100%) was 100% (Table 5).

To ensure these results and 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 28.32% (25.73%-31.06%) (Table 5). On the other hand, the PCR analysis showed 80 HCV positives and 1307 negatives (Table 3) with overall percent agreement % (95% CI) of 5.77% (4.66%-7.12%) and positive percent agreement % (95% CI) of 100% (95.42%-100%). Importantly, the PPV of this analysis was 5.77% (4.66%-7.12%) (Table 5).

As a final confirmation of our diagnostic analysis, we compared the PCR and INNOLIA results. Of 1097 samples, 78 HCV positives, 791 negatives, and 288 indeterminate were detected in both assays. However, 228 samples showed positive results in INNOLIA but were negative in PCR (Table 4). The overall percent agreement and positive percent agreement (95% CI) between both assays were 79.22% (76.72%-81.52%) and 25.49% (20.93%-30.66%), respectively. Notably, the positive predictive value and the negative predictive values were both 100% (99.52%-100%) (Table 5).

Table 1:Comparison between ARCHITECT anti-HCV round 1 and round 2

ARCHITECT anti-HCV round 2	Positive	Negative
ARCHITECT anti-HCV round 1		
Positive	1393	0
Negative	0	0

Table 2: Comparison between ARCHITECT anti-HCV round 2 and INNO-LIA HCV

INNO-LIA HCV	Positive	Negative	Indetermined
ARCHITECT anti-HCV round 2			
Positive	311	787	295
Negative	0	0	0

Table 3: Comparison between ARCHITECT anti-HCV round 2 and PCR

PCR	Positive	Negative
ARCHITECT anti-HCV round 2		
Positive	80	1307
Negative	0	0

Table 4: Comparison between INNO-LIA HCV and PCR

INNO-LIA HCV			
PCR	Positive	Negative	Indetermined
Positive	78	0	2
Negative	228	791	288

Table 5: Performance agreement of HCV detection between different methods

	Overall Percent Agreement % (95% Cl)	Positive Percent Agreement % (95% CI)	Negative Percent Agreement % (95% CI)	Positive Predictive Value % (95% CI)
ARCHITECT Anti-HCV Round 1 & ARCHITECT Anti-HCV Round 2	1393/1393 100% (99.72%-100%)	1393/1393 100% (99.72%-100%)	N/A	N/A
ARCHITECT Anti-HCV Round 2 & PCR	80/1387 5.77% (4.66%-7.12%)	80/80 100% (95.42%-100%)	N/A	80/1387 5.77% (4.66%-7.12%)
ARCHITECT Anti-HCV Round 2 & INNO-LIA HCV	311/1098 28.32% (25.73%-31.06%)	311/311 100% (98.78%-100%)	N/A	311/1098 28.32% (25.73%-31.06%)
PCR & INNO-LIA HCV	869/1097 79.22% (76.72%-81.52%)	78/306 25.49% (20.93%-30.66%)	791/791 100% (99.52%-100%)	78/78 100% (95.31%-100%)

Order of reference standard
INNO-LIA HCV > PCR > ARCHITECT Anti-HCV Round 2 > ARCHITECT Anti-HCV Round 1

5.2 Evaluation of the performance agreement in different HBV diagnostic methods

For HBV data analysis, we used a similar strategy as HCV. The data was obtained from 1061 HBV samples that were subjected to three successive rounds of screening named round 1, rerun 1, and rerun 2, respectively. These runs were done on the same ARCHITECT analyzer (A) and by the same technician. All samples were HBV positive in round 1 of screening, whereas in rerun 1, 1051 samples were positive and 10 were HBV negative (Table 6). Comparing those two rounds, the percent agreement analysis showed an overall percent agreement of 99.06% (95%CI; 99.64-100%) and a positive percent agreement of 100% (95%CI; 99.64-100%) (Table 10). On the other hand, the comparison between round 1 and rerun 2 showed 1060 HBV positive samples and only one negative sample (Table 7) with a 99.91% agreement (overall and positive percent agreements; 95% CI, 100% (99.64%-100%) (Table 10). Based on these results, HBV positive patients were recalled again for blood collection and another run was performed on another ARCHITECT analyzer (B) by an alternative technician (round 2). Consistent with the results obtained in round 1 and rerun 2 (Table 7), round 2 of HBV screening showed 1060 positive samples and one negative sample with 100% agreement (Table 8 and Table 10).

To further confirm these results, the recollected samples from round 2 were subjected to another diagnostic test, HBsAg confirmatory test (neutralization test). In this analysis, both assays showed 712 HBV positive samples and one negative sample for HBV. However, there was a discrepancy in 384 samples that were positive in round 2 but negative in the HBsAg confirmatory test (Table 9). This yielded an overall percent agreement of 67.20% (64.32%-69.96%). Finally, the positive percent agreement of 100% (99.46%-100%) (Table 10).

Table 6:Comparison between ARCHITECT HBsAg round 1, rerun 1 and round 1

ARCHITECT HBsAg round 1, rerun 1		
ARCHITECT HBsAg round 1	Positive	Negative
Positive	1051	10
Negative	0	0

Table 7:Comparison between ARCHITECT HBsAg round 1, rerun 2 and round 1

ARCHITECT HBsAg round 1, rerun 2		
ARCHITECT HBsAg round 1	Positive	Negative
Positive	1060	1
Negative	0	0

Table 8: Comparison between ARCHITECT HBsAg round 1 and round 2

ARCHITECT HBsAg round 1	Positive	Negative
ARCHITECT HBsAg round 2		
Positive	1060	1
Negative	0	0

Table 9: Comparison between ARCHITECT HBsAg confirmatory and round 2

ARCHITECT HBsAg confirmatory	Positive	Negative
ARCHITECT HBsAg round 2 final positive		
Positive	712	348
Negative	0	1

Table 10: Performance agreement of HBV detection between different methods

	Overall Percent Agreement % (95% CI)	Positive Percent Agreement % (95% CI)	Negative Percent Agreement % (95% CI)	Positive Predictive Value % (95% CI)
ARCHITECT HBsAg Round 1 & ARCHITECT HBsAg Round 1 Rerun 1	1051/1061 99.06% (98.28%-99.49%)	1051/1051 100% (99.64%-100%)	N/A	N/A
ARCHITECT HBsAg Round 1 & ARCHITECT HBsAg Round 1 Rerun 2	1060/1061 99.91% (99.48%-99.98%)	1060/1060 100% (99.64%-100%)	N/A	N/A
ARCHITECT HBsAg Round 1 & ARCHITECT HBsAg Round 2	1060/1061 99.91% (99.48%-99.98%)	1060/1060 100% (99.64%-100%)	N/A	N/A
ARCHITECT HBsAg Round 2 Final Positive & ARCHITECT HBsAg Confirmatory	713/1061 67.20% (64.32%-69.96%)	712/712 100% (99.46%-100%)	1/349 0.00% (0.00%-0.02%)	712/1060 67.17% (64.29%-69.93%)

Order of reference standard ARCHITECT HBsAg Confirmatory > ARCHITECT HBsAg Round 2 > ARCHITECT HBsAg Round 1

CHAPTER 6: DISCUSSION

In Medical Health Commission, the reliability, sensitivity, and reproducibility of HCV and HBV diagnostic assays are of significant importance. This is because the results obtained from these assays determine the fate of the examined individual (46). In Qatar, individuals tested positive for HCV or HBV are not allowed to stay in the country and thus their job offer usually gets withdrawn by their employer. HCV diagnostic scheme starts by detecting viral antibodies using different immunoassays and then followed by viral RNA detection to confirm positive cases using RT-PCR (23). Thus, the initial anti-HCV antibodies screening using those immunoassays is considered a crucial step in HCV diagnosis. Among those assays, ELISA based assays stand out as the most common method used in different labs around the world to primarily detect HCV infection (47, 48).

However, false-positive results with low titer samples in HCV screening is considered a fundamental and common problem in many labs. Indeed, the false HCV positive cases range between 15%-60%, with an average of 35% among populations where HCV prevalence is less than 10% (48-50). Therefore, in this study, we aimed to assess the reproducibility and efficiency of our diagnostic HCV protocol in the Qatar Medical Health Commission laboratory. We did several comparisons between different Architect instruments as well as between different confirmatory diagnostic methods.

Our results showed a 100% agreement in the results obtained from two different Architect instruments and performed by two different technicians as presented by overall percent agreement and positive percent agreement of 100% (Table 5). These results indicated the reliability and high reproducibility of our initial screening method using an Architect. This indicates that the Medical Commission's testing platform (Architect analyzer) give precise and reproducible results.

Also, The Medical Commission's protocol implemented in the pre-analytical phase regarding the applicant's identification is a perfect one. Our data is supported by other reports that confirmed the diagnostic reliability of Architect in HCV diagnosis. These studies also affirm the high specificity and sensitivity of this assay that reaches up to 100% (51-54). Moreover, our results strongly agree with a large study conducted by Jonas et al 2005, who used ARCHITECT immunoassay analyzer from different centers in Germany, Sweden, and Switzerland and confirmed the high specificity, sensitivity, and reliability of the assay in HCV detection (55).

Interestingly, Architect results did not show a high percent agreement with those of PCR (overall percent agreement and PPV is 5.77%) indicating that 5.77% of all Architect anti-HCV positive samples have current HCV infection. This discrepancy could be due to the inability of PCR assay to detect the viral RNA during the transient control phase of viral replication by the immune system. Furthermore, in nonviremic patients, no HCV RNA is detected using PCR, whereas anti-HCV antibodies could be easily detected by Architect in sera samples collected from those patients (56, 57). This could indicate that these patients might resolved HCV infection. In this case, the individual has to be called again for PCR detection after two weeks. Therefore, an HCV Ag assay would be an optimal solution to be used as the first confirmatory test preceding the PCR step (58, 59). This would be a more reliable, cost-effective, specific, and sensitive first confirmatory test as compared to PCR. Similar to the results obtained for the overall percent agreement of the ARCHITECT Round 2 and INNO-LIA, the PPV was 28.32%. This indicates 28.3% of all Architect anti-HCV positive samples have confirmed HCV antibodies which indicates that these 28.3% either having current, chronic or resolved HCV infection, which can be differentiated by HCV PCR.

The overall percent agreement and positive percent agreement (95% CI) between both assays were 79.22% (76.72%-81.52%) and 25.49% (20.93%-30.66%), respectively. This indicates that 25.5% of all confirmed HCV antibody test (INNOLIA HCV), having current HCV infection while 74.5% having past resolved infection. Also, the table shows that the Negative Percent Agreement is 100% (791/791) indicating that all Architect ELISA HCV positive samples which were not confirmed by antibody HCV confirmatory test were HCV PCR negative (i.e. not having HCV infection). Also the table shows 2 out of 290 INNO LIA HCV indeterminate samples having current HCV infection (HCV PCR Positive). An indeterminate HCV antibody confirmatory test indicates that the individual might be in the seroconversion period, delayed appearance of full specific antibodies, or nonspecific reaction. Such individual needs to be tested in 4 weeks to determine the definitive HCV antibody status. These two positive PCR results might indicate either these two individuals in the seroconversion period or there is delayed appearance of full specific antibodies.

Our HBV results also showed the high reproducibility and reliability of the Architect assay as indicated by the 100% overall percent agreement and positive percent agreements between the different runs of the test as well as different Architect analyzers (Tables 7 and 8). Our data is in a strong agreement with another study that compared the results obtained from three different analyzers (Abbott Architect i2000 Assay, the Roche Modular Analytics E170 Assay, and an Immunoradiometric Assay) to detect HBV antibodies and showed that the Architect i2000 showed the highest agreement of 97.3% (60). Yet, the overall percent agreement of 67.20% (64.32%-69.96%) indicating that 67 % of all Architect HBsAg test positive samples have current HBV infection. Also, 33% (348/1060) are unconfirmed by HBsAg confirmatory test. These unconfirmed HBsAg positive screening test results should be interpreted in

conjunction with test results of other HBV serological markers such as Hepatitis B core antibody "total and IgM" and hepatitis B surface antibody before considering the initial screening test is a false positive. Repeat testing is recommended at a later date along with HBV DNA PCR, if clinically indicated.

6.1 Limitations of the study

Similar to any other study, our study had one main limitation. In this study, time was too limited to analyze the enormous amount of data collected during the research period. Therefore, we were not able to confirm the negative or non-reactive cases. Hence, we were not able to calculate the sensitivity and specificity due to the absence of the negative data.

6.2 Future Implications

Future studies can be done to confirm the negative or non-reactive cases while having enough time to analyze the data. Moreover, since our data showed that there was no advantage gained from repeating Architect analysis. We would recommend to healthcare facilities in Qatar to use Architect only one time. In this manner, we would be able to successfully save our limited resources, while reducing cost and time for future testing procedures.

CHAPTER 7: CONCLUSION

In conclusion, Architect HBV and HCV can detect all positive cases, so if the case is identified as negative by the test, then it is negative. However, if the case was identified as positive by Architect HBV, then there is a 32.7% chance that this is false, and it is negative. On the other hand, if the case identified as positive by Architect HCV, then there is a 77.6% chance that this is false, and it is negative. Based on these conclusions, it is recommended to use Architect one time, if it is negative then there is no need for further testing. If it is positive then in almost three-quarters of the cases, it will be negative, hence, PCR can be done for confirmation. If PCR is positive, then there is no need for more tests and the case can be identified as positive. If it is negative in a third of cases, the case will be positive and INNOLIA can be done in this case to confirm the positive status.

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APPENDIX

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 Architect and confirmatory test for the diagnosis of HCV and HBV infection"*. The Principal Investigator, Ms. Reham 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**} <u>unless</u> (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,

Noudin Fallouch

on behalf of

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Appendix B

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

February 27th, 2020

Dr. Hadi Yassine
Biomedical Research Center
Qatar University
Phone: +974 4403 6819
Email: hyassine@qu.edu.qa

Dear Dr. Hadi Yassine,

Sub.: Research Ethics Review Exemption
Project Title: "Performance evaluation of Architect and confirmatory test for the diagnosis of HCV and HBV infection"

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_ 26.220, QU-IRB Checklist HMY 2.2.20, MC-MOPH TEST PROPOSAL HMY BRC FEB2020, MoPH Approval letter (Reham 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 by 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 1243-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

Institutional Review Board
(IRB)
Office Of Academic Research

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