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Improving Hepatitis B diagnosis by implementing statistical quality controls

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Abstract

Hepatitis B is a viral infection that causes major health problems worldwide. The hepatitis B virus (HBV) and 1.2 million deaths per year globally infect more than two billion people. Hepatitis B mainly affects the liver and causes liver cirrhosis and hypocellularity carcinoma. This study investigates diagnostic quality parameters of quantitative polymerase Chain Reaction (qPCR) and enzyme-linked Immunosorbent Assay (ELISA) called enzyme immunoassay (EIA) for HBV Diagnosis. The research was conducted at the Molecular Biochemistry Laboratory (MBL) in the Department of Biochemistry at the University of Agriculture, Faisalabad, Pakistan. The samples for the randomized study containing 977 male and female samples who had pre-diagnosis HBV infection were selected for this comparative study and collected from the Punjab Institute of Nuclear Medicine (PINUM) Hospital and Allied Hospital Faisalabad, Pakistan. This study compared the sensitivity and specificity of the ELISA assay with the HBV qPCR assay to diagnose HBV infection. The cut-off value < 1.00 was considered non-reactive, whereas ≥ 1.00 was reactive patients for ELISA. Similarly, HBV viral load was measured on qPCR < 10^1 IU/mL was considered a negative result, and > 10^1 IU/mL showed positive results. Statistical data analysis was conducted using Statistical Package for Social Sciences (SPSS 22) software. These tests were used to diagnose HBV infection on a large scale to calculate the percentage value of sensitivity, specificity, and other parameters. With a 95% confidence interval, the qPCR's positive predictive value (PPV) was 46%, whereas 52% for ELISA. The negative predictive value (NPV) for qPCR was 98%, but it was the same for ELISA. The specificity for qPCR was 91% and 92% for ELISA. The sensitivity value for qPCR was 83%, and 87% for ELISA was observed from the samples. Accuracy for both methods varied, which showed their proficiency and size. The results obtained from qPCR were more accurate and authentic than those obtained from ELISA.

Keywords: HBV, Diagnosis, ELISA, qPCR, statistical quality controls

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1. Introduction

Hepatitis B is a viral infection that is recognized as a severe and major health problem all over the world. Globally, more than 350 million individuals are recurrently infected with HBV, and 15-25% of them cause the threat of emerging and declining HBV-related prolonged liver diseases comprising cirrhosis and hepatocellular carcinoma [1]. HBV is a member of the Hepadnaviridae family with partially double-stranded circular DNA. The external surface of the HBV virus is coated with lipoprotein, and the size of the hepatitis B surface antigen (HBsAg) is 42 nm long [2]. The Hepatitis virus may transmit from one infected individual to another in various ways, including blood and other body fluids. Tribal tattooing and scarification, sexual contact, blood transfusions, hazardous injection practices, injectable drug use, and professional exposure of health care professionals are all frequent routes of transmission. HBV damages the liver and causes acute and chronic hepatitis as well as hepatomegaly and Hepatocellular Carcinoma (HCC) [3]. The HBV can replicate in the plasma, saliva, breast milk, vaginal secretion, semen, and ascetic liquid [4]. The World Health Organization (WHO) released global recommendations for preventing, caring for, and treating persons from chronic HBV infection in March 2015 [5].

HBV DNA levels in clinical practice have a good sensitivity and dynamic range. To minimize residual contamination, real-time PCR of HBV-DNA is conducted in a closed system, approved for *in vitro* diagnostics (IVD). Standard techniques were used to ensure the accuracy of diagnostic analysis [6]. With a 95% confidence interval, each sample can be calculated for accuracy, linearity, negative likelihood ratio (NLR), precision, positive likelihood ratio (PLR), specificity, and sensitivity. The SPSS software is commonly used to analyze the data statistically [7]. Over the last decade, antiviral treatment for hepatitis B showed significantly that viral repetition could now remain successfully inhibited in 95 percent of chronic Hepatitis B patients [8]. Despite evidence showing that initial treatment and viral destruction substantially lower the threat of evolution to hepatomegaly, liver cancer, and eventual mortality, active treatment rates remain relatively low once diagnosed accurately [9]. Passive vaccination can be done with mutual blood from people who have improved impulsively from severe HBV and have high antigen (HBsAg) levels [10]. Hepatitis B disease eradication needs five main treatments, according to the worldwide strategy: hepatitis B vaccination, inhibition of HBV transmission from mother to kids, plasma and injection protection, harm reduction programming for drug users, and enhanced testing and treatment [11].

2. Methodology

The study was conducted at the Molecular Biochemistry Laboratory (MBL) in the Department of Biochemistry at the University of Agriculture, Faisalabad, Pakistan.

2.1 Sample collection

The samples for the randomized study containing 977 males and females who had pre-diagnosed HBV infection were selected for this comparative study. These samples were collected from the PINUM Hospital, Faisalabad and Allied Hospital, Faisalabad, Pakistan, from June to September 2021.

2.1.1 Inclusion criteria

The samples were collected from male and female individuals with a pre-diagnosis of HBV infection visiting the designated hospitals.

2.1.2 Exclusion criteria

The HBV samples without diagnosis and those collected from another hospital were excluded from this study.

2.2 Ethical consideration

All the selected applicants were informed and signed the consent form to use their information for the research analysis, and the ethical committee approved it as well.

2.3 Sample Collection and Detection of HBV

All collected samples centrifuged to separate serum and stored at -20 to -80 ^{o}C with the assigned codes until testing. This study compared the sensitivity and specificity of a modified third-generation ELISA and qPCR assays. HBV antigen was assessed using the Abnova ELISA kit on the stored samples. The cut-off value < 1.00 was considered non-reactive, whereas ≥ 1.00 was reactive patients for ELISA. Similarly, HBV viral load was measured on qPCR < 10^1 IU/mL was considered a negative result, and > 10^1 IU/mL showed positive results.

2.4 Statistical analysis

Standard procedures were used for determining the accuracy of diagnostic analysis. For each sample, parameters of accuracy, linearity, negative likelihood ratio (NLR), precision, positive likelihood ratio (PLR), specificity, and sensitivity with a confidence interval of 95% were calculated [12]. Statistical data analysis was conducted using Statistical Package for Social Sciences (SPSS 22) software.

2.4.1 Sensitivity

Sensitivity, also known as true positive rate, is the probability of a positive test result if a medical test has a high true positive rate. The sensitivity of the test was calculated by the number of truly diseased patients divided by all diseased patients [13].

2.4.2 Specificity

Specificity, or true negative rate, is the probability of a patient not having an illness testing negative. In other words, it measures how well a test can decrease false positives. The specificity is the number of non-diseased patients divided by the total number of non-diseased patients [13].

2.4.3 Accuracy

The accuracy of a test is its ability to differentiate the patient and healthy cases correctly. To estimate the accuracy of a test, the proportion of true positives and true negatives was calculated in all evaluated cases [13].

2.4.4 Positive and Negative Predictive Value

Positive predictive value relates to the chance of a positive test leading to an illness, whereas negative predictive value refers to the likelihood of a negative. As a result of the medical test findings, these values are highly important in clinical settings since they give doctors an indicator of the likelihood of sickness or an event, such as death [13].

2.5.4.1 Positive predictive value (PPV)

Percentage of the positive patients with a positive test who have a disease. It tells us about the result and how many tests are positive from predictive samples.

2.5.4.2 Negative predictive value (NPV)

NPV is the percentage of patients with a negative test who do not have the disease. It tells us about the result and how many tests are negatives from predictive samples.

3. Results

3.1 HBV Diagnosis by qPCR

977 samples were subjected to quantitative PCR (qPCR) for the detection of HBV DNA. Among 977 samples, 80 showed positive results, whereas 897 subjects were declared negative. Further evaluation of 897 samples by

qPCR revealed that 77 were positive and indicated the presence of infection, and 820 tested negative, with no indication of infection (Table 3.1). Tests were applied to the 80 participants infected by HBV. All of the positive 80 samples were diseased and actual participants. Among 80 participants, 67 were positive, and 13 were negative. Figure 3.1 compares the percentage (%) and the actual test.

3.1.1 Data interpretation of qPCR

Out of 977, 144 (14.7%) tests were positive, and 833 (85.3%) were normal participants or true negative. Out of these 144, 77 were correctly positive, and 67 were correctly false positive. Among 833 non-diseased participants, 820 were found negative, and 13 were found correctly false negative.

3.1.2 Quality parameters for qPCR

After the statistical analysis of qPCR results, estimated values of different quality parameters (sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, accuracy, positive predictive value, negative predictive value, and disease prevalence) are shown in Table 3.2. The sensitivity of qPCR was 83.75 percent, and specificity was calculated as 91.41 percent.

3.2 Enzyme immunoassay

About 977 HBV samples were tested by ELISA to detect HBV antigens. After analysis, 81 showed positive results, whereas 896 subjects were declared negative. Further evaluation of 896 samples by ELISA revealed that 63 were positive and indicated the presence of infection, and 833 tested negative, with no indication of infection. Among the 81 participants, only 10 were normal people, and 71 showed the infection positively. Hepatitis B is a viral infection that causes major health problems worldwide. It belongs to the Hepadnaviridae family, with circular double-stranded DNA. HBV and almost 378 Million chronic transporters infect more than two billion people globally. The infection is transmitted directly through the blood and other body fluids of contaminated individuals. HBV stabbings the liver and causes penetrating hepatitis and persistent infections like Chronic Hepatitis B (CHB), Cirrhosis, and Hepatocellular Carcinoma (HCC) [3]. After analyzing data from the Faisalabad area of Punjab, Pakistan, it was estimated that the virus more afflicts people between the ages of 20 and 50 than older and teenage groups.

The main goal of antiviral treatments is to inhibit HBV infection and reduce liver diseases [14]. Various HBV infection detection screening programs are needed to provide a good picture of the prevalence of HBV infection in Pakistan's general population. It is also possible that an unidentified vector is involved in its spread to the healthy population. The detection of HBV DNA was done using the most accurate technique for diagnosis. HBV infection was tested by qPCR and ELISA assay, resulting in false positive or false negative findings. According to the Punjab population survey 2021, total HBV-positive cases in Faisalabad were 20.93%. After collecting these samples for the detection of HBV, the most accurate and authentic diagnostic tools with high specificity, accuracy, and sensitivity values are used. The 977 samples were tested by qPCR and ELISA to diagnose HBV DNA. After testing, results showed positive and negative subjects.

According to the findings, 91.8 percent of suspected HBV patients tested negative for HBV qPCR, whereas 8.2 percent tested positive. When these samples were retested using qPCR, 85.3 percent were found to be HBV-DNA negative, whereas 14.7 percent were found to be HBV-DNA positive. After analysis, the estimated positive predictive values were 46.5 percent, the estimated negative predictive values were 98.4 percent, and the estimated sensitivity and specificity values were 83.7 and 91.4 percent, respectively. Similarly, HBV ELISA testing revealed that 91.7 percent of patients were negative and 8.3 percent were positive. When the same samples were retested using ELISA, 86.3 percent were negative A, and 13.7 percent were positive. After analysis, the estimated positive predictive values were 52.9 percent, the estimated negative predictive values were 98.8 percent, and the estimated sensitivity and specificity values were 87.6% and 92.9 percent, respectively.

A lack of awareness about the risk factors involved in its transmission/spread and medical malpractice, such as reusing syringes and using non-sterilized or improperly sterilized medical equipment, could be the cause for the high prevalence of HBV in this densely populated area, which is largely comprised of uneducated people. HBsAg is present in the body's blood, saliva, breast milk, and vaginal secretions [15]. A broad-based HBV immunization program is now being used or implemented in most nations [16]. It has shown that immunization can reduce the prevalence of HBV and HCC in children, even at this young age. Vaccination against hepatitis B (HBV) is considered the peak of cancer treatment. HBV vaccine is recommended for anyone who has been in contact with blood or blood products, is planning a trip to a developing country, lives with a chronically infected person, works in a health-care setting with patient contact, was born to HBsAg-positive mothers, or suffers from chronic liver disease [17].

3.2.1 Data interpretation ELISA

After testing, some individuals were diseased, and the remaining were normal. 977 tests were done, out of which 134 (13.7%) were diseased patients or positive tests, and 843 (86.3%) were normal participants or true negative. Out of these 134, 63 were positive, and 71 were false positive. The test was applied to 843 normal individuals, a percentage value of 91.7%, and when the test was applied to diseased patients, the total percentage was 8.3% (Table 3.3).

3.2.2 Quality parameters for ELISA

Statistical analysis of ELISA results, which shows different parameters and estimated values, \ given in Table 3.4. Parameters like sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, accuracy, positive predictive value, negative predictive value, and disease prevalence analysed in Table 3.4.

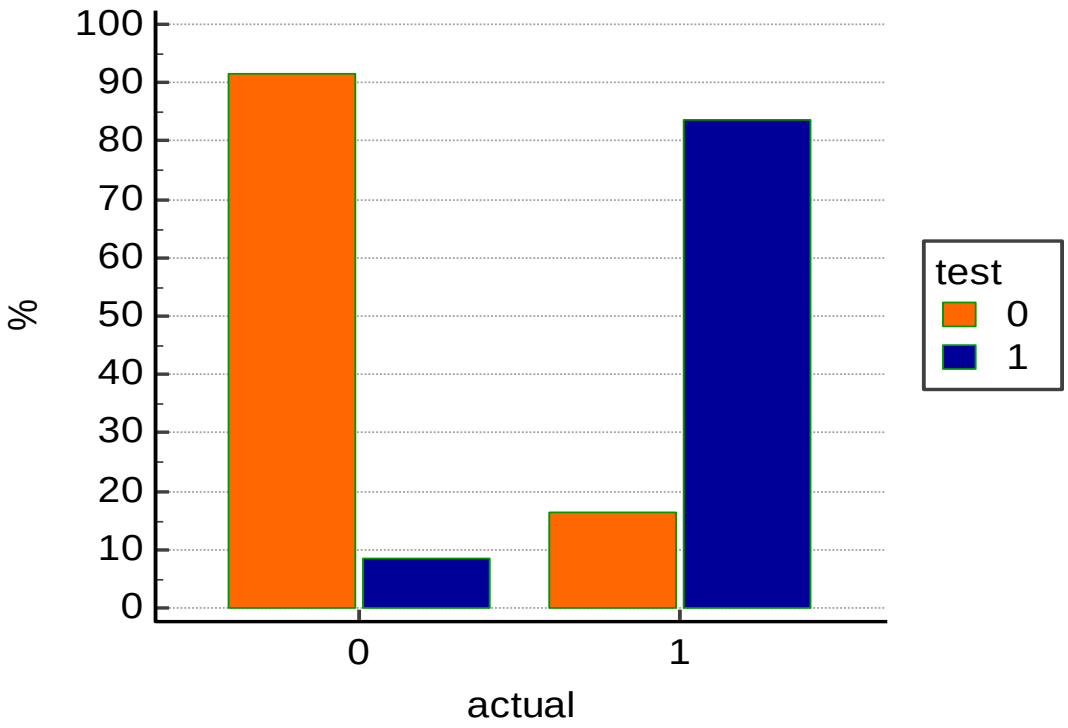

Figure 3.1. The figure shows the results of two testing groups used for the analysis. Group (0) indicates the ratio of controlled or normal subjects (orange colored bar), out of which some positive subjects (who were infected) were detected upon further testing (shown with blue colored bar). The second group (1) is the set of infected patients (blue bar), out of which the presence of some negative subjects (orange bar) with no infection detected.

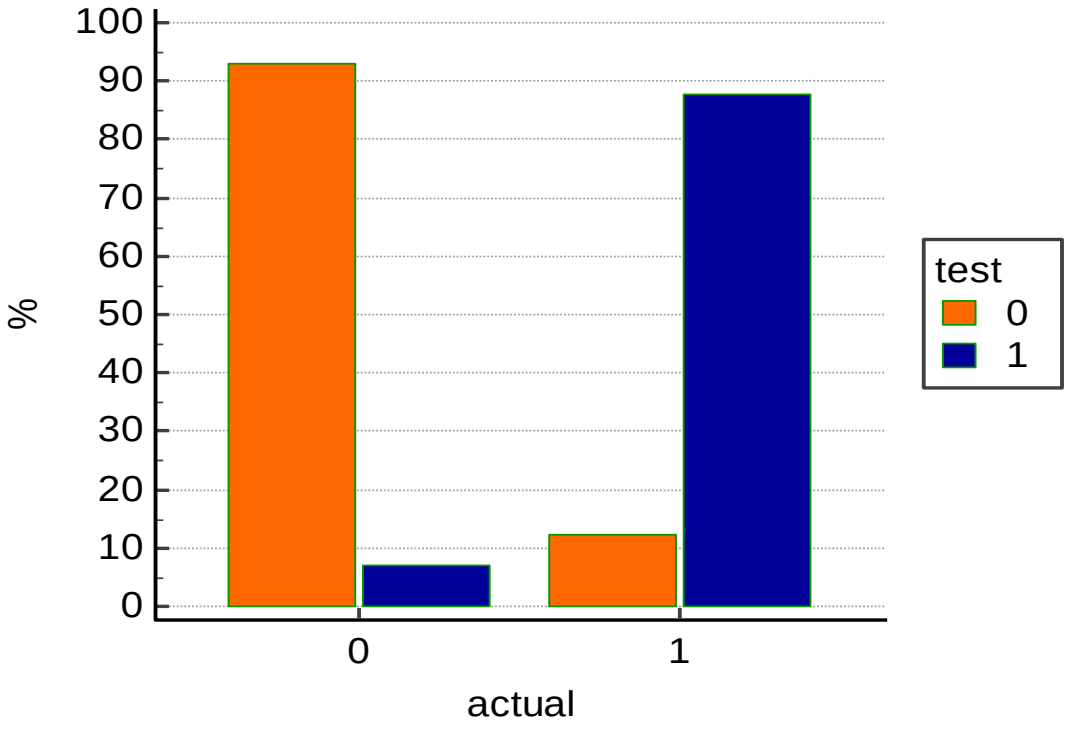

Figure 3.2. The results of two testing groups were used for the analysis. Group (0) indicates the ratio of controlled or normal subjects (orange colored bar), out of which some positive subjects (who were infected) were detected upon further testing (shown with blue colored bar). The second group (1) is the set of infected patients (blue bar), out of which the presence of some negative subjects (orange bar) with no infection detected.

Table 3.1: HBV Test Results through quantitative PCR

Actual	Test		Percentage (%)
	Negative 0	Positive 1	
Negative 0	820	77	91.8%
Positive 1	13	67	8.2%
Total	833 (85.3%)	144 (14.7%)	977

Table 3.2: Statistical analysis of qPCR and percent occurrence of HBV patients

Parameters	Estimate	Interval Estimate
Sensitivity	83.750%	73.816% to 91.054%
Specificity	91.416%	89.388% to 93.166%
Area Under Curve	0.876	0.854 to 0.896
Positive Likelihood Ratio	9.756	7.718 to 12.333
Negative Likelihood Ratio	0.178	0.108 to 0.292
Disease prevalence	8.188%	6.546% to 10.088%
Positive Predictive Value	46.528%	40.770% to 52.379%
Negative Predictive Value	98.439%	97.458% to 99.046%
Accuracy	90.788%	88.798% to 92.528

Table 3.3: HBV Test Results using enzyme immunoassay

Actual	Test		Percentage (%)
	Negative 0	Positive 1	
Negative 0	833	63	896 (91.7%)
Positive 1	10	71	81 (8.3%)
Total	843 (86.3%)	134 (13.7%)	977

Table 3.4: Statistical analysis of ELISA and percent occurrence of HBV disease in patients

Parameters	Estimate	Interval Estimate
Sensitivity	87.654%	78.466% to 93.918%
Specificity	92.992%	91.123% to 94.573%
AUC	0.903	0.883 to 0.921
Positive Likelihood Ratio	12.508	9.724 to 16.089
Negative Likelihood Ratio	0.133	0.074 to 0.237
Disease prevalence	8.265%	6.618% to 10.168%
Positive Predictive Value	52.985%	46.699% to 59.177%
Negative Predictive Value	98.818%	97.907% to 99.335%
Accuracy	92.551%	90.725% to 94.116%

4. Conclusion

Sensitivity and specificity are important measures of a diagnostic test's accuracy; they cannot be used to predict a person's likelihood of being unwell. The prevalence of infection influences positive and Negative predictive values. The graphs were created to provide a workable method for comprehending the distribution of individuals. The investigation demonstrated that qPCR yielded more accurate results than ELISA did. While the HBV virus is dormant and produces false-positive results, ELISA detects the antigen; in contrast, qPCR measures the viral load in your bloodstream in IU/mL. This figure indicates the high and low viral load.

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Conflict of interest

The authors state no conflict of interest.

Authors Contribution

Shahid Nadeem: Data Curation, Investigation, Resources, Methodology, Funding, and writing original draft.
Amer Jamil, Awais Altaf, Muhammad Arif: Conceptualization, Supervision, editing, and proofreading.
Syed Ali Raza Shah, Farwa Batool, Munazza Shaheen, Faiza Akram, and **Sana Malik:** Sampling and resources.
Muhammad Arif: Statistical Analysis.

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