



## Improving Hepatitis c diagnosis by implementing statistical quality control

***Farwa Batool<sup>1</sup>, Awais Altaf<sup>2\*</sup>, Muhammad Idrees Jilani<sup>3</sup>, Imran Riaz Malik<sup>4</sup>, Shahid Nadeem<sup>1</sup>, Sameer Hassan<sup>2</sup>, Muhammad Arif<sup>4</sup>, Amer Jamil<sup>1\*</sup>***

<sup>1</sup>*Molecular Biochemistry Lab., Department of Biochemistry, University of Agriculture, Faisalabad, Punjab, Pakistan.*

<sup>2</sup>*Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan.*

<sup>3</sup>*Department of Chemistry, The University of Lahore, Lahore, Pakistan.*

<sup>4</sup>*Department of Biotechnology, University of Sargodha, Sargodha, Pakistan.*

<sup>5</sup>*Department of Mathematics and Statistics, University of Agriculture, Faisalabad, Punjab, Pakistan.*

### Abstract

Hepatitis C (HCV) is the RNA-enveloped virus belonging to the flavivirus family, causing severe hepatic problems worldwide. While screening for HCV, many results are false negative or false positive, reaching chronic stages of the infection and eventually cancer. This study aimed to estimate various parameters like sensitivity, specificity, accuracy, and likelihood ratios for HCV by reverse transcriptions-quantitative Polymerase Chain Reaction (RT-qPCR) and Enzyme-linked Immunosorbent Assay (ELISA). This study was conducted at the Molecular Biochemistry Laboratory (MBL), University of Agriculture Faisalabad, Pakistan. Four cities (Gojra, Samundri, Toba and Kamalia) of Faisalabad were targeted. Samples of the participants were obtained from the PINUM Cancer Hospital and Allied Hospital Faisalabad. One thousand one hundred male and female participants pre-diagnosed with HCV infection were selected for this comparative study. All collected samples were centrifuged to separate serum and stored at -20 °C to -80 °C with the assigned codes until testing. This study compared the sensitivity and specificity of a modified third-generation ELISA kit with the SYSTAAQ HCV RT-qPCR assay. HCV antibodies were assessed with the Abnova hepatitis C virus Ab ELISA kit on the stored samples. According to the manufacturer's instructions, the cut-off index < 1.000 was considered NON-REACTIVE or negative and  $\geq 1.000$  is considered reactive or positive. HCV viral load was measured on real-time PCR of  $< 10^1$  IU/mL were considered as low levels of virus and  $> 10^1$  IU/mL showed positive results, indicating the presence of the virus. SPSS version 22.0 for Windows was used for the statistical summary and data analysis. The results of specificity and sensitivity were obtained in the form of percentages. The community benefits from statistical quality control in hepatitis C diagnosis by promoting trust in the healthcare system, assuring accurate and reliable testing, improving treatment results, and increasing diagnostic effectiveness. All parameters for both diagnostic tools were compared. The sensitivity for HCV-RNA PCR was 96%, but for ELISA, it was 57%. The specificity for ELISA was 95%, whereas 98% for HCV-RNA PCR. The positive predictive value (PPV) of the HCV RNA was calculated as 80% for ELISA and 94% for RT-qPCR. Accuracy for both results also varied, showing their efficiency and capacity. Results showed that HCV-RNA PCR was quite accurate and more reliable than ELISA. ELISA detects the HCV antibody while the HCV virus remains inactive, pertaining to false positive or negative results. In contrast, PCR can detect HCV's presence, absence and load in subjects' blood more accurately and sensitively.

**Keywords:** HCV, RNA, PCR, ELISA, Sensitivity, Accuracy

**Full length article** \*Corresponding Authors, e-mail: [awaisaltaf362@yahoo.com](mailto:awaisaltaf362@yahoo.com); [amerjamil@yahoo.com](mailto:amerjamil@yahoo.com)

### 1. Introduction

Hepatitis C (HCV) is the most prevalent deadly virus globally, with various genotypes. HCV is an enveloped RNA virus, i.e. single-stranded and small in size, belonging to the flavivirus family with a high genetic diversity [1-2]. It is the only member in the hepacivirus genus identified in 1989 [3]. A huge nucleotide diversity was reported in the isolates after discovering the HCV virus [4-5].

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HCV has been classified into 11 genotypes (assigned as 1-11) that differ in their nucleotide sequences from 30% to 50%, six of which are major genotypes [6-7]. The distribution of the genotypes and subtypes of HCV varies geographically [8]. Almost 170 million people are infected with the HCV virus globally [9]. HCV is the third major reason of death due to liver cancer worldwide.

The etiology of prevalence is unidentified individuals in the early stages [10]. Without any therapy, acute HCV turns into chronic HCV, increasing the risk of liver diseases leading to carcinoma and complete failure [11]. The transfer rate of HCV is increasing rapidly, and the reason for HCV prevalence in developing countries is the poor diagnosis and limited accessibility of expensive quantitative assays [12]. Therefore, the quantitation of HCV RNA load in serum or plasma tests can underrate the absolute circulating viral load [13]. The gaps in the diagnosis of HCV can be eliminated with efficient strategies like expanding the accessible and less expensive diagnosis and treatment [13]. HCV qPCR should be performed if screening is positive [14]. The problem with the screening for HCV is that many of the results are declared as false positive and false negative even if they are HCV positive, which eventually leads to the chronic stages of the disease in the form of cancer [15-16]. Hence, improving the procedure to identify HCV-positive individuals effectively and directing them to proper treatment can decrease the risk of severe issues, thus gradually helping to eliminate the deadly virus. The study doesn't look into the underlying causes of false-negative and false-positive HCV screening findings nor look into viable solutions to increase diagnostic precision and boost the efficacy of HCV screening techniques.

## 2. Materials and Methods

### 2.1. Place of Study

This study was conducted at the Molecular Biochemistry Laboratory of the University of Agriculture Faisalabad, Faisalabad. Four cities (Gojra, Samundri, Toba and Kamalia) of Faisalabad were targeted. Samples of the participants were obtained from the PINUM Cancer Hospital and Allied Hospital Faisalabad.

### 2.2. Sample size

One thousand one hundred male and female participants were selected, fulfilling the inclusion and exclusion criteria for this comparative study.

#### 2.2.1. Inclusion Criteria

- Participants with a pre-diagnosis of HCV infection.
- Male and female participants.
- Participants from the specified cities (Gojra, Samundri, Toba, and Kamalia) of Faisalabad.
- Samples were obtained from the designated hospitals (PINUM Cancer Hospital and Allied Hospital Faisalabad).

#### 2.2.2. Exclusion Criteria

- Participants without a pre-diagnosis of HCV infection.
- Participants outside the specified target cities.
- Samples were obtained from sources other than the designated hospitals.

### 2.3. Ethical considerations

All the selected patients were informed and signed the consent form to use their information for the research analysis, and the Ethical Committee also approved it.

### 2.4. Sample Collection Technique

All collected samples were centrifuged to separate serum and stored at  $-20^{\circ}\text{C}$  with the assigned codes until testing. This study compared the sensitivity and specificity of a modified third-generation ELISA kit with the SYSTAAQ HCV RT-qPCR assay. HCV antibodies were assessed with the Abnova hepatitis C virus Ab ELISA kit on the stored samples. According to the manufacturer's instructions, a cut-off index of  $< 1.000$  is non-reactive or negative, and  $\geq 1.000$  is reactive or positive. According to the manufacturer's instructions, HCV viral load was measured on real-time PCR of  $< 10^1$  IU/mL were considered as low levels of virus and  $> 10^1$  IU/mL showed positive results, indicating the presence of the virus.

### 2.5. Statistical Analysis

SPSS version 22.0 for Windows was used for the statistical summary and data analysis. The results of specificity and sensitivity were obtained in the form of percentages.

Cross-tabulation (2x2) of true disease status and test results were used to calculate the test performance measures. The sensitivity of both tests was calculated as the total number of true positive test results divided by the sum of false negative and true positive test results. Specificity was calculated as the number of true negative test results divided by the sum of true negative and false positive test results. Negative predictive values (NPV) were calculated as the number of true negatives divided by the sum of false negative and true negative test results. Positive predictive values (PPV) were calculated as the number of true positives divided by the sum of true positive and false positive test results. NPV and PPV are affected by the prevalence of the disease, whereas NPV showed a reverse relation with PPV. Hence, PPV shows an inverse relation with NPV.

## 3. Results

Out of the 1100 HCV suspects after the antibody testing by ELISA, 249 (23.8%) were infected, and 799 (76.2%) were healthy individuals. These results were divided into two groups, i.e. infected and healthy subjects. Antibody testing with the same procedure was performed again on 249 infected patients; 144 were positive, and 105 were negative (uninfected). Another group of 799 healthy individuals showed positive results for 36 (whereas negatives for 763, declaring them uninfected). Overall, the percentage ratio of positive results in both groups was 17.2%, and negative was 82.8%. The same samples of 1100 subjects were tested by RT-qPCR diagnostic method to detect HCV RNA. Of all, 796 (76.2%) were sero-negative, and 249 (23.8%) were sero-positives. There were some expected chances of sero-positives among the seronegative results.

Upon further testing of 796 sero-negatives, 14 plasma samples were sero-positive, and 782 were declared

uninfected. Many individuals were still expected to be HCV RNA negatives even in the sero-positive results. In further testing of HCV RNA in 249 sero-positive, 9 were negative, and 240 were positive. However, the negative results obtained by the molecular diagnostic methods applied to positive results were considered false-positive, whereas positive results declared among the negative results were considered false negatives. The overall sensitivity of RT-qPCR and ELISA was calculated as 96.386% and 57.831%, with specificity of 98.241% and 95.494%, respectively. The sensitivity and specificity of both these molecular diagnostic tests were not similar. All the other parameters for both diagnostic methods, such as positive and negative likelihood ratios, PPV, NPV, accuracy and disease prevalence, showed different values in Table 1. The accuracy for both differed, showing 97.80% and 86.54%, respectively. A comparison of negative and positive testing ratios by ELISA and by RT-qPCR is given in Figure 1 and Figure 2.

ELISA result 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 was detected. Figure 2 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 was detected.

#### 4. Discussion

Infection with the hepatitis C virus (HCV) is problematic. It is the third main cause of cancer-causing deaths worldwide. Many infected individuals are unaware of their condition because of asymptomatic conditions in the early stages [17-18]. Without any therapy, acute HCV develops into chronic HCV, increasing the risk of liver diseases and leading to carcinoma and complete failure. Usually, severe complications or death may occur in individuals with long-term infections turned into cirrhosis, which can develop in 15-20% of patients [19]. HCV can be spread through careless blood transfusions, including using contaminated instruments and unsterilized needles during medical care globally. One less efficient route of infection is

blood exposure to the mucus membrane [20]. Besides the liver, the complications can be extrahepatic, as diverse as lymphoma, diabetes, and kidney diseases. The detection of HCV can be done in breast milk, saliva, semen and other fluids that are not considered efficient modes of transmission [21]. The HCV diagnostic assays available are expensive and out of reach, so their standardization is crucial. However, a small population has access to HCV RNA tests, leaving a huge ratio of undiagnosed individuals [13]. The problem with the screening for HCV is that many of the results have resulted in false negatives, even if they are HCV positive, pertaining to the chronic stages, i.e. cancer [22].

The major issue of HCV prevalence in populated areas is the unawareness of its disasters and types. Any unknown factor may be involved in the prevalence of the virus. Hence, the improvement in the procedure to identify HCV-positive individuals effectively and direct them to treatment centers can decrease the risk of severe issues, thus helping in the gradual elimination of deadly viruses [23-24]. For the estimation of the ratios and inspection of HCV tests, this study was conducted for which samples from the live patients were collected, and the procedure of RT-qPCR and ELISA was applied to them [25]. The percentage ratio for the positive testing was 24.3%, and the healthy was 75.5% in RT-qPCR testing.

In comparison, ELISA showed a 17.2% percentage ratio for positive testing, and healthy testing was 82.8%. This number determines whether the viral load is high or low [26]. The collected data and results were compared with the help of a statistical tool, SPSS 22. Diagnostic test's sensitivity, specificity, positive predictive and negative predictive values are the words that are used to characterize the capacity of a test to determine whether a person is healthy or infected [27].

It is emphasized that while sensitivity and specificity are significant indicators of a diagnostic test's accuracy, they are useless in assisting clinicians in estimating the likelihood of illness in particular patients [28-30]. Even though predictive values may be used to estimate the risk of disease, both prognostic and predictive values. This means using predictive values generated for one group to another with a different disease prevalence would be inaccurate. Medical resources, diagnosis, and treatment must improve in developing countries. There are limited resources available: lack of access to medical and health resources about the disease to the patients, limited knowledge and training, and awareness about the disease. The training should be conducted to improve the health literacy and how to access the medical resources for patients in Pakistan [31-37].

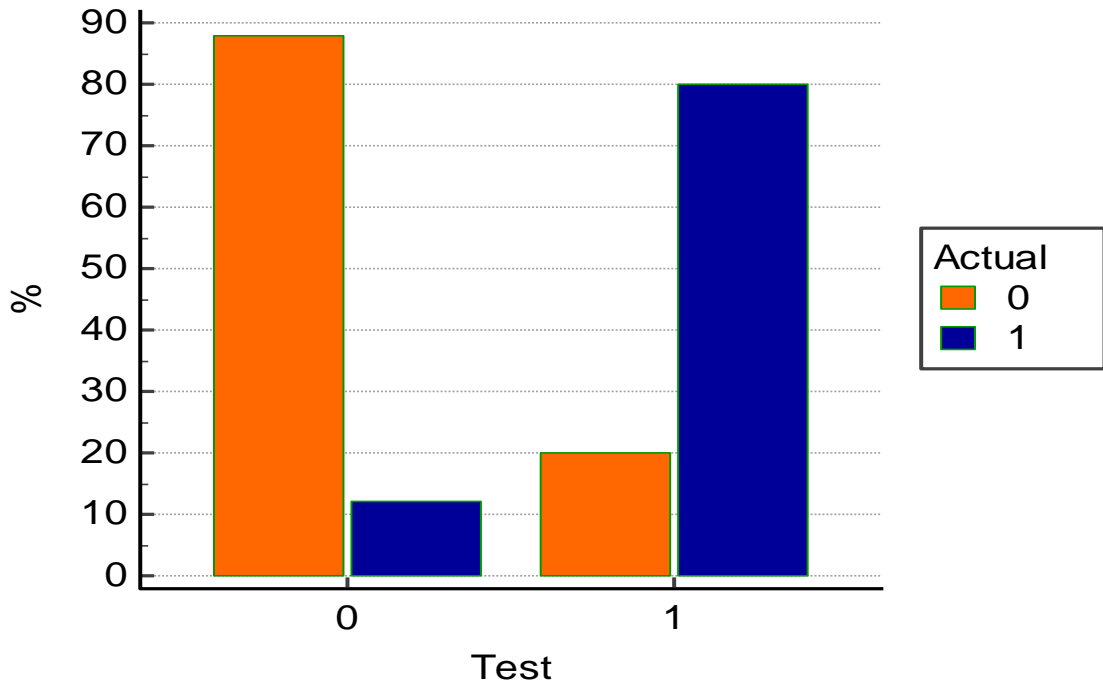


Figure 1: Statistical comparison of the data obtained from Enzyme-linked Immunosorbent Assay (ELISA).

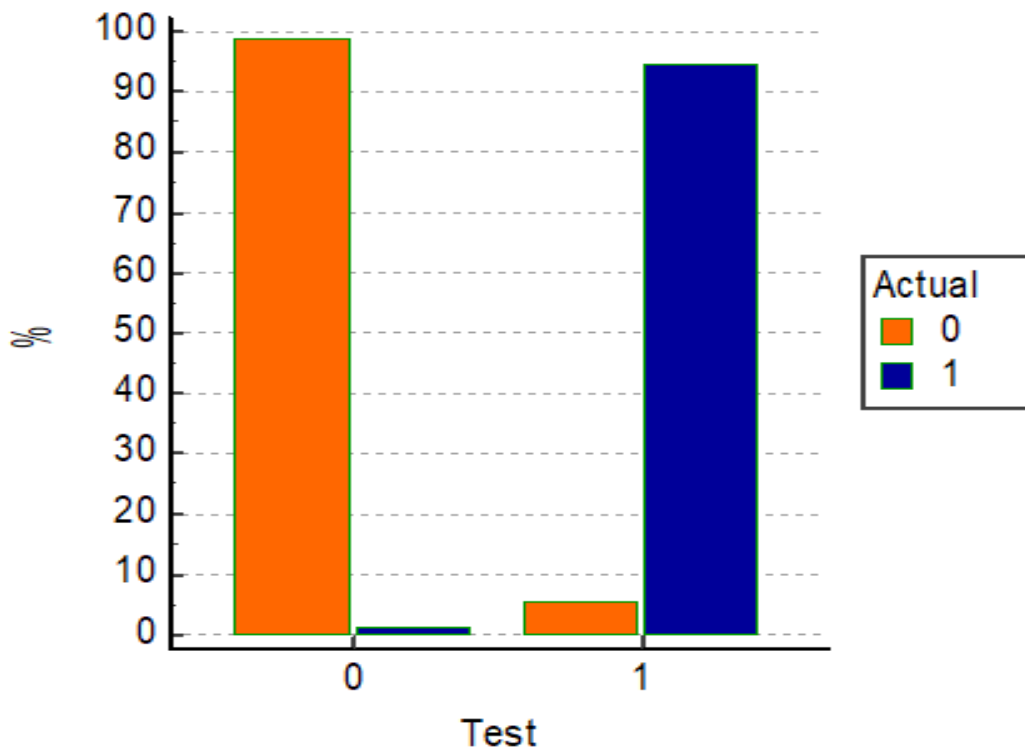


Figure 2: Statistical comparison of the data obtained from reverse transcriptions-quantitative Polymerase Chain Reaction (RT-qPCR).

**Table 1:** Sensitivity, Specificity, likelihood ratios, predictive values, prevalence and accuracy for RT-PCR and ELISA calculated from the areas of Faisalabad.

Parameters	Estimate for RT-PCR	Interval (RT-PCR)	Estimate for ELISA	Interval (ELISA)
Sensitivity	96.39%	93.25 to 98.33%	57.83%	51.43 to 64.04%
Specificity	98.24%	97.07 to 99.04%	95.49%	93.82 to 96.83%
AUC	0.97	0.96 to 0.98	0.77	0.74 to 0.79
Positive Likelihood Ratio	54.80	32.59 to 92.16	12.83	9.17 to 17.97
Negative Likelihood Ratio	0.04	0.02 to 0.07	0.44	0.38 to 0.51
Disease prevalence	23.83%	21.27 to 26.53%	23.76%	21.21 to 26.46%
Positive Predictive Value	94.49%	91.07 to 96.65%	80.00%	74.08 to 84.85%
Negative Predictive Value	98.86%	97.86 to 99.40%	87.90%	86.26 to 89.37%
Accuracy	97.80%	96.72 to 98.60%	86.54%	84.33 to 88.56%

**RT-qPCR:** Reverse transcriptions-quantitative Polymerase Chain Reaction; **ELISA:** Enzyme-linked Immunosorbent Assay; **AUC:** Area under curve;

## 5. Conclusions

Sensitivity and specificity are significant indicators of a diagnostic test's accuracy, but they cannot be utilized to expect the likelihood of illness of an individual. Positive and negative predictive values depend on the prevalence of infection. The graphs were made for the feasible approach towards understanding the ratio among the people. The study showed that the results of RT-qPCR were more accurate than ELISA. ELISA detects the antibody while the HCV virus remains inactive with false positive results, whereas RT-qPCR can detect the viral load in your bloodstream in IU/mL. This number determines whether the viral load is high or low.

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### Conflict of interest

The authors state no conflict of interest.

### Authors Contribution

**Frawa Batool:** Data Curation, Investigation, Resources, Methodology, Funding and writing original draft.

**Amer Jamil and Awais Altaf:** Conceptualization, Supervision and Validation.

**Muhammad Idrees jilani and Imran Riaz Malik:** Writing review and Editing.

**Sameer Hassan and Shahid Nadeem:** Sampling and resources.

**Muhammad Arif:** Formal Analysis.

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