

HCV Antibody ELISA Kit

96 Tests Kit
Enzyme Immunoassay for the
Detection of Hepatitis C Virus Antibody
(For In Vitro Diagnostic Use Only)
Catalogue No. PT-HCV-96

PISHTAZ TEB DIAGNOSTIC

Introduction

Hepatitis C Virus (HCV) is a member of Flaviviridae family and has been introduced at 1989. HCV is responsible for post-transfusion hepatitis which is formerly known as non-A Non B hepatitis. The viral genome consists of a single stranded RNA (~1000 Nucleotides) which is associated with matrix, capsid and surrounded by an envelope. The HCV genome is coding for a precursor polyprotein which is cleaved after translation into structural (Core, E1, E2) and nonstructural (NS1, NS2, NS3, NS4, NS5) proteins. Genetically the virus can be categorized into 6 major genotypes and at least 80 serotypes. Studies have been shown that the most prevalent genotypes in countries like Iran are 1a, 3a, 1b and 4 respectively and 1% of population are infected. The major modes of virus transmission are blood transfusion, blood products and contaminated injections. It is also transmitted by contaminated tattooing, ear piercing, circumcision and hemodialysis. Prior to implementation of specific HCV virus screening in blood transfusion tests, the virus was responsible for 90% of post transfusion hepatitis. There are also few reports of vertical (mother to fetus) as well as sexually transmitted cases. Many of affected individuals with acute HCV infection are asymptomatic or with mild symptoms of fatigue, nausea and jaundice. The disease incubation period varies from 15-150 days. At least 80% of infected persons will develop chronic disease. Cirrhosis occurs in 20-50% of chronic HCV patients after a period of 20 years and the annual incidence of hepatocellular carcinoma in cirrhotics is approximately 3%. Detection of specific antibody against particular antigens of HCV is critical for the diagnosis of infected patients. Among various methods which are exploited for the detection of HCV antibody,

ELISA test offers reliability and high sensitivity particularly in preliminary and screening diagnosis. Positive samples should be rechecked and confirmed by Western blot analysis.

This kit has third generation HCV ELISA format with high sensitivity and specificity and detects specific antibody against HCV.

Test Principle

The test principle is based on indirect enzyme immunoassay. Micro titer wells are coated with certain amount of HCV recombinant antigens including NS3, NS4, NS5 and CORE antigens. Then serum samples are allowed to react with solid phase antigens. If HCV-specific antibodies (IgG and IgM) are presented in the serum they will bind to HCV antigens through their individual Fab section. After incubation, the wells are washed to remove unbound antibodies and anti-human antibodies (IgG/IgM) conjugated with HRP is added into the wells following another incubation and wash step. A solution of TMB is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of specific anti-HCV is directly proportional to the color intensity of the test sample.

Materials Provided With Kit

1. Antigen coated plate. (1 plate, 96 wells)
Microtiter wells coated with HCV recombinant antigens.
2. Sample diluents: A PBS buffer containing 0.02% Tween 20, protein as stabilizer and 0.05% Kathon CG as preservative. 1 red vial, 25 ml.
3. Enzyme Conjugate (20X): monoclonal Antihuman IgG/IgM-HRP conjugated. 1 vial, 0.75 ml, Should be diluted 1:20 with conjugate diluent before use.
4. Conjugate diluent: Phosphate buffer solution containing protein as stabilizer and 0.05% Kathon CG as preservative. 1 green vial, 15 ml.
5. Positive control: A buffer containing inactivated positive (anti-HCV antibody) human serum, 0.05% Kathon CG as preservative and protein as stabilizer, 1 red vial, 2 ml.
6. Negative control: A buffer containing inactivated negative (anti-HCV antibody negative) human serum,

- 0.05% Kathon CG as preservative and protein as stabilizer, 1 yellow vial, 5 ml.
- Wash solution: Phosphate Buffer Saline (PBS) containing 0.05 % Tween 20 as detergent and Kathon CG as preservative 1 vial, 50 ml (20X).
 - Chromogen-substrate: contains Tetramethyl benzidine and hydrogen peroxide. 1 vial, 12 ml, ready to use.
 - Stop solution: 1 molar hydrochloric acid solution 12 ml.
 - Cardboard sealer.

- Kit should be stored at 2-8°C upon receipt and when it is not in use.
- Keep Un-used wells in their sealed bag with desiccants. Opened kits are stable for 4 months.
- Reagents stability are marked on their labels. Please ensure that do not use expired date reagents.

Materials/Equipment required but not provided with Test Kit

- ELISA reader with 450 and 630 nm (reference filter)
- Precision pipettes 10, 100 and 200 µl
- 37°C water bath or incubator
- 5% Sodium hypochlorite solution
- Distilled water
- Disposable pipette tips
- Absorbent paper

General Information

- Do not mix kit reagents from different lot numbers. All kit components must be used only in original kit.
- This kit is just for the detection of Anti-HCV antibody in human serum and plasma.
- All reagents obtained from human sources are negative for HBs Ag, HCV Ab and HCV Ag. The positive control contains HCV antibody, and is inactivated by incubation at 56°C for 30 minutes. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.
- Positive sera, wash solution residuals and equipments suspected to contamination by HCV, should be disinfected by 5% hypochlorite solution for 30 minutes or autoclaved at 121°C for 60 minutes.

Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. Do not use diluted or pooled sera/plasmas. If testing cannot be done within an hour after sample collection, refrigerate the specimen (maximum 1 week at 2-8°C) and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20°C. Avoid freeze-thaw of specimen during storage. Samples suspected to microbial contamination should not be used.

Reagents & Specimens Preparation

- All reagents should be allowed to reach room temperature (22-28°C) before use.
- Working wash solution: dilute concentrated wash solution 1:20 with distilled water before use.
- Test steps should be done sequentially.
- Working enzyme conjugate: Dilute the concentrated conjugate with the conjugate diluent according to table 1. Mix it gently. This working solution remains stable for 4 weeks at 2-8°C.

TABLE 1

Strips required	1	2	3	4	5	6
Conjugate diluent ml	0.95	1.9	2.85	3.8	4.75	5.7
Concentrated conjugate ml	0.05	0.1	0.15	0.2	0.25	0.3
Strips required	7	8	9	10	11	12
Conjugate diluent ml	6.65	7.6	8.55	9.5	10.45	11.4
Concentrated conjugate ml	0.35	0.4	0.45	0.5	0.55	0.6

Storage Conditions

Assay Procedure

1. Use required number of wells and keep the remaining with desiccant in tightly closed sealed bag. Consider first well as **Blank** and next two wells for **Positive** and **Negative controls**.
2. Add 200 µl of sample diluent into each well except blank, positive and negative controls.
3. Add 200 µl of positive and negative controls. Add 10 µl of test serums into their related wells.
4. Seal the plate with cardboard sealer tightly. Mix gently for 15 seconds. Leave wells for 60 minutes at 37°C. (water bath/incubator)
5. Remove the well's content by flicking plate's contents into a waste container. Rinse and flick the microtiter wells 5 times (each with 300 µl of working wash solution).
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Add 100 µl of working enzyme conjugate into each well except blank.
8. Cover the plate with cardboard sealer tightly. Leave wells for 30 minutes at 37°C.
9. Repeat step 5 and 6.
10. Dispense 100 µl of chromogen/substrate to each well.
11. Incubate the microplate wells at room temperature and dark for 15 minutes to develop color.
12. Add 100 µl of stop solution to the wells to stop reaction.
13. Read absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available) reference filter is highly recommended.

procedure. In such a case, check the efficiency of the washing device.

3. The mean OD value of positive control is higher than the 1.1 and lower values indicate kit reagents decay. In such a case, check expiry date of the kit before repeating the assay.

Result Calculation

1. Measure absorbance of controls and samples at 450 nm (Use 630 nm filter as reference filter if it's available).
2. To calculate cut off value for the test, use following formulae:

$$\text{Cut-off} = \text{Negative control mean OD } 450 \text{ nm} + 0.2$$

3. To determine positive and negative results the S/Co index:

$$S/Co = \frac{\text{Sample OD}}{\text{Cut-off value}}$$

can be used. Based on this index, results equal/higher than 1.0 should be considered as positive and results less than 1.0 are considered as negative.

4. Negative results indicate absence of or undetectable antibody response against HCV antigens in early stages of infection.
5. Positive results should be rechecked and those which become negative should be reported as negative.
6. Faulty washing and sampling errors may lead to false positive results.
7. In case of recheck with positive results, confirmatory tests like Western blot analysis or PCR should be done.

Validity of the Assay

The assay is to be considered valid if:

1. The OD of the blank well is lower than 0.1 and higher values indicate chromogen/substrate contamination. In such a case, repeat the assay carefully and check the reagent.
2. The OD value for the negative control is lower than 0.15 and higher values indicate an incorrect washing

Performance Characteristics

- Sensitivity

To evaluate test sensitivity, BBI panels were used. They included:

1. Sero-conversion panels

Results using these panels are shown in Table 2 A and B.

TABLE 2 A.

Sample ID	Panel name			
	BBI PHV 901		BBI PHV 908	
	PT HCV KIT S/Co	Ortho 3.0 int. S/Co	PT HCV KIT S/Co	Ortho 3.0 FDA Li. S/Co
1	0.5	0.0	0.0	0.0
2	0.3	0.0	0.2	0.0
3	4.9	5.9	0.2	0.0
4	3.8	6.0	1.8	0.1
5	4.4	6.1	2.7	0.3
6	3.9	6.0	5.4	1.7
7	5.8	>9.1	6.8	4.9
8	5.1	7.4	7.6	4.9
9	5.6	>9.1	9.2	>5.0
10	4.9	9.1	9.8	>5.0
11	5.3	>9.1	9.8	>5.0
12			10.9	>5.0
13			10.2	>5.0

TABLE 2 B.

Sample ID	Panel name					
	BBI PHV 914		BBI PHV 907		BBI PHV 909	
	PT HCV KIT S/Co	Ortho 3.0 FDA Li. S/Co	PT HCV KIT S/Co	Ortho 3.0 int. S/Co	PT HCV KIT S/Co	Ortho 3.0 S/Co
1	0.0	0.0	0.3	0.0	0.2	0.0
2	0.0	0.0	0.2	0.0	1.2	1.3
3	0.0	0.0	0.2	0.0	1.1	1.3
4	0.2	0.0	1.3	0.1		
5	0.6	0.2	3.3	0.4		
6	0.8	0.3	3.2	1.0		
7	2.3	3.2	3.7	4.4		
8	2.5	>4.7				
9	3.1	>4.7				

2. Worldwide anti-HCV performance (WWHV301) panel. This panel includes 18 positive and 2 negative members which were detected by this kit precisely.

Sample ID	PT HCV KIT S/Co	Ortho 3.0 int. S/Co	Sample ID	PT HCV KIT S/Co	Ortho 3.0 FDA Li. S/Co
1	5.8	>5.0	11	1.8	>5.0
2	5.9	>5.0	12	5.5	>5.0
3	5.8	>5.0	13	5.2	>5.0
4	5.8	>5.0	14	6	>5.0
5	0.1	0.0	15	3.9	>5.0
6	4.0	>5.0	16	3.9	>5.0
7	1.5	>5.0	17	2.5	>5.0
8	0.3	0.1	18	3.3	>5.0
9	3.8	>5.0	19	1.6	>5.0
10	5.4	>5.0	20	2.0	>5.0

For further estimation of sensitivity, 97 positive samples which are already confirmed by Western blot analysis were tested by PT HCV Antibody ELISA kit and all samples were positive. Our results are comparable to other commercial HCV Ab kits and displayed 100% sensitivity.

- Specificity

To verify test specificity, 2000 random serum/plasma samples were tested with this kit. Results showed 5 positive samples which on re-check 3 samples remained positive and 2 became negative. The estimated specificity of kit is 99.5-100%.

- Interferences

Results obtained from known serum samples with HIV, ANA and RF antibodies as well as hemolytic and icteric samples are shown in table 3.

TABLE 3

Serum type	No. of samples tested	Result
ANA positive	14	Negative
Anti-HIV Positive	31	Negative
RF Positive	15	Negative
Hemolytic Serum	8	Negative
Lipemic Serum	7	Negative

- Precision

Within and Between-run precision tests were performed on positive and negative serum controls and also on a positive serum. Results are shown in table 4 and 5.

TABLE 4. WITHIN ASSAY PRECISION

Serum type	Test repeat	Mean OD	SD	CV%
Positive control	20	1.68	0.026	1.55
Negative control	20	0.06	0.007	11.7
Positive serum	20	0.31	0.014	4.51

TABLE 5. BETWEEN ASSAY PRECISION

Serum type	Test repeat	Mean OD	SD	CV%
Positive control	10	1.73	0.05	2.9
Negative control	10	0.05	0.006	12.0
Positive serum	10	0.31	0.01	3.2

All samples were run in duplicate.




Enzyme Immunoassay Results: An Important Predictor of Low Likelihood of Hepatitis C Infection. *Clinical Chemistry* 2003; 49:479-486.

8. Hepatitis C Virus in Iran: Epidemiology of an Emerging Infection. *Arch Iranian Med* 2005; 8:84-90.
9. Genetic Heterogeneity of Hepatitis Virus and its Clinical Significance. *Current Drug Targets-Inflammation & Allergy* 2005; 4:47-55.

References




1. Guidelines for Laboratory Testing and Result Reporting of Antibody to Hepatitis C Virus: Centers for Disease Control and Prevention. *MMWR* 2003; 52(No.RR-3).
2. Evaluation of a Rapid Assay as an Alternative to Conventional Enzyme Immunoassay for detection of Hepatitis C Virus-Specific Antibody. *Journal of Clinical Microbiology* 2005; 43:1977-1978.
3. Are the Real HCV Infection Features in Iranian Patients the Same As What Is Expected? *Hepatitis Monthly* 2005; 5:3-5.
4. Significance of Indeterminate Third-Generation Hepatitis C Virus Recombinant Immunoblot Assay. *Journal of Clinical Microbiology* 1996; 34:80-83.
5. Development of Simple and Highly Sensitive Enzyme Immunoassay for Hepatitis C Virus Core Antigen. *Journal of Clinical Microbiology* 1999; 37:1802-1808.
6. Laboratory Assay for Diagnosis and Management of Hepatitis C Virus Infection. *Journal of Clinical Microbiology* 2002; 40:4407-4412.
7. Low-Positive Anti-Hepatitis C Virus

HCV ELISA Test Procedure

Stop Solution	100 μ l 	100 μ l 	100 μ l 
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Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).

Step 1




Reagent	Blank	Serum Control	Sample
			
Sample Diluent	None	None	200 μ l
Serum Control (Pos/Neg)	None	200 μ l	None
Sample	None	None	10 μ l

Cover the microplate wells with cardboard sealer tightly, Mix gently for 15 seconds and incubate them for 60 minutes at 37°C.


Step 2

Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.


Step 3




Enzyme conjugate	None 	100 μ l 	100 μ l 
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Cover the microplate wells with cardboard sealer and incubate for 30 minutes at 37°C.

Step 4

Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.


Step 5

Chromogen-substrate solution	100 μ l 	100 μ l 	100 μ l 
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Incubate wells for 15 minutes at room temperature and dark.


Step 6