Size : 274 x 218 mm

Qualisa™

Microwell Enzyme Immunoassay | HCV

Enzyme Linked Immunosorbent Assay (ELISA) for detection of antibodies to HCV in human serum or plasma.
3rd Generation Assay
FOR IN VITRO DIAGNOSTIC USE ONLY
Store at 2°C to 8°C

INTENDED USE

QUALISA™ HCV is a 3rd Generation EIA & is intended to be used for the detection of antibodies to Hepatitis C Virus in human serum or plasma.

SUMMARY AND EXPLANATION

Hepatitis C Virus (HCV) is now known to be the major cause of parenterally transmitted non-A, non-B hepatitis. Until the virus was characterized, diagnosis was made by exclusion of all other known causes of hepatitis. Antibody to HCV is found in over 80% of patients with well documented non-A, non-B hepatitis. The worldwide prevalence of HCV is 0.2 to 2% in blood donors and up to 80% in intravenous-drug users. Seroepidemiologic studies show that the seroprevalence of HCV infection in India varies between 0.3% to 11.3%. In a large percentage of HCV cases, transmission is by transfusion and other parenteral means such as sharing of needles, occupational exposure to blood and hemodialysis. However, in case of half of HCV infections, the route of transmission is unknown. HCV establishes a chronic infection in 50 to 80% of cases. Chronic infection is often asymptomatic even in the presence of liver damage discernible on biopsy. Chronic HCV is characterized by fluctuating alanine aminotransferase (ALT or SGPT) levels and recognizable changes in liver histology. Chronic infection can lead to cirrhosis and hepatocellular carcinoma..

PRINCIPLE OF THE ASSAY

QUALISA™ HCV is an Enzyme Linked Immunosorbent Assay (ELISA) that employs highly purified recombinant antigens representing most conserved antigenic segments of Core, NS3, NS4 and NS5 antigens from multiple genotypes. These antigens are so selected that they recognize all six major genotypes of HCV prevalent worldwide.
Microwell strips are coated with recombinant antigens representing Core, NS3, NS4 and NS5 antigens. Samples along with positive and negative controls are added in the coated wells and incubated. The wells are washed to remove unbound components and anti-human IgG conjugated to horseradish peroxidase (HRPO) is added. After a short incubation the wells are washed again and bound enzyme is detected by adding substrate. The reaction is stopped after specified time with acid and absorbance is determined for each well at 450 nm with an ELISA reader. The cutoff value (COV) is calculated by the given formula and absorbance of all the wells are compared with the COV. Any sample having absorbance more than the COV is considered reactive.

KIT COMPONENTS

QUALISA™ HCV has following components:

1. Coated microwells :Microwells coated with recombinant antigens representing HCV derived from multiple genotypes of HCV. Ready to use. 96 Wells : (3x8) x 4 pouches
   192 Wells : (3x8) x 8 pouches
   480 Wells : (3x8) x 20 pouches
2. Positive control: Inactivated and stabilized human serum reactive for HCV and non-reactive for HIV & HBsAg with preservatives.
3. Negative control: Inactivated and stabilized human serum non reactive for HIV-1 & HIV-2, HBsAg and HCV.
4. Conjugate: Goat anti-human IgG-HRPO conjugate (50 X). To be diluted 50 times with conjugate diluent.
5. Conjugate diluent: Buffered solution containing stabilizing proteins and preservatives.
6. Sample diluent: Buffered solution containing stabilizing proteins and preservatives. Ready to use.
7. Substrate: Solution containing Tetramethyl benzidine (TMB) and hydrogen peroxide. Ready to use.
8. Wash buffer: Buffer contains surfactants (20 X). To be diluted 20 times with distilled or deionized water.
9. Stop solution: Diluted Sulphuric acid. Ready to use.
10. Microwell holder.
11. Instruction for use.
12. ELISA protocol sheet.
13. Plate sealer.

STORAGE AND STABILITY

1. QUALISA™ HCV kit is stable at 2-8°C upto the expiry date printed on the label.
2. Coated microwells should be used within one month of opening the pouch. Each pouch contains 3 strips of 8 wells. Any unused well(s) should be resealed with cellotape (and desiccant pouch) to prevent moisture absorption and stored at 2-8°C for future use.
3. In case the desiccant pouch changes colour from blue to white, the strips should not be used.
4. Diluted conjugate must be used immediately.
5. Diluted wash buffer is stable upto one week.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Manual or automatic pipette
2. Pipette tips
3. Glass or polypropylene container for conjugate dilution
4. Absorbent sheets
5. ELISA washer
6. ELISA reader
7. Pipetting troughs or boats
8. Disinfectant
9. Reagent grade water
10. Disposable gloves
11. Timer
12. Biohazard waste container
13. Serological pipettes

Size : 137 x 218 mm

SAMPLE COLLECTION

(1). No prior preparation of the patient is required. (2).Collect blood specimen by venipuncture according to the standard procedure. (3). Serum or plasma can be used. (4).Specimen should be free of particulate matter and microbial contamination. (5). Preferably use fresh sample. However, specimen can be stored refrigerated for short duration. For long storage, freeze at -20°C or below. Do not freeze samples in frost-free freezer. (6).Specimen should not be frozen and thawed repeatedly. (7).Do not heat inactivate before use.(8).Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.

PRECAUTIONS

(1). Bring all reagents and specimen to room temperature before use. (2). Do not pipette any material by mouth. (3).Do not eat, drink or smoke in the area where testing is done. (4).Use protective clothing and wear gloves when handling samples. (5). Use absorbent sheet to cover the working area. (6). Immediately clean up any spills with sodium hypochlorite. (7).Dispose off all the reagents and material used as if they contain infectious agent. (8).Neutralize acid containing waste before adding hypochlorite. (9).Do not use kit after the expiration date. (10).Do not mix components of one kit with another. (11). Always use new tip for each specimen and reagent. (12). Do not let the dispensing tip of ELISA washer touch liquid in the wells. (13). Do not allow liquid from one well to mix with other wells. (14).Do not let the strips dry in between steps. (15).Incubation times and temperatures other than those specified may give erroneous results. (16). Cross contamination of reagents or samples may give false results. Samples must remain confined to microwells during testing. (17).Inadequate removal of residual wash buffer can cause inconsistent color development. Microwell strips should be tapped vigorously and blotted on absorbent paper or towels to minimize residual wash buffer. (18).Reusable glassware must be washed and thoroughly rinsed free of detergents prior to use. All glassware must be clean and dry before use. (19).Adequate washing of wells is extremely important. Inadequately washed wells will exhibit high background values, and may show false positive values. For manual washing, aspirate the contents of the wells, then fill each well with wash buffer solution. Avoid cross- contamination of the wells, particularly in the first wash after aspiration. Drain all of the wash buffer from the wells by inverting, then shaking residual wash buffer from the wells with a sharp "snapping" motion of the wrist. Repeat these steps for at least six washes. The wells should then be tapped vigorously on a paper towel or other absorbent material to remove all traces of residual wash buffer. The use of an automated microwell washing system will assure consistent washing of the wells, and is recommended.

REAGENT PREPARATION

1. Dilute wash buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water).
2. Dilute conjugate 50 times (for example add 20 µl concentrated conjugate to 980µl conjugate diluent).

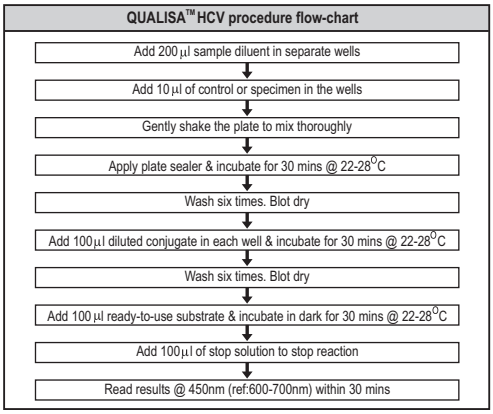
No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
50 x Conjugate	20µl	40µl	60µl	80µl	100µl	120µl	140µl	160µl	180µl	200µl	220µl	240µl
Conjugate Diluent	980µl	1960µl	2940µl	3920µl	4900µl	5880µl	6860µl	7840µl	8820µl	9800µl	10780µl	11760µl

TEST PROCEDURE

1. Bring all the reagents and specimen to room temperature before use.
2. Take out required number of strips and immediately close the pouch.
3. Prepare ELISA protocol sheet indicating the location of controls and specimen.
4. Use controls in duplicate.
5. Add **200 µl** sample diluent to separate wells.
6. Add **10 µl** of control or specimen in the wells.
7. Gently shake the plate to mix thoroughly. Care should be taken to avoid spillage.
8. Apply plate sealer and incubate for 30 minutes at 22-28°C.
9. Wash each well by filling approximately **350 µl** diluted wash buffer and aspirating/flicking off six times. Blot dry.
10. Add **100 µl** diluted conjugate in each well and incubate for 30 minutes at 22-28°C.
11. Wash six times as in step 9. Blot dry.
12. Add **100 µl** substrate and incubate at 22-28°C away from light for 30 minutes.
13. Stop reaction by adding **100 µl** stop solution. The stop solution should be added in the same sequence as substrate addition.
14. Read the absorbance at 450 nm with 600-700 nm as reference within 30 minutes of stopping the reaction.

RUN CRITERIA

1. The individual absorbance value of negative controls should be less than 0.1.
2. The individual absorbance value of positive controls should be more than 1.0.



CALCULATIONS

The cut-off value (COV) is calculated by adding 0.3 to average absorbance value of negative control.

EXAMPLE

Absorbance of Negative Control (NC)	
Reading-1	0.020
Reading-2	0.014
Average NC reading	0.017

COV = Av.NC + 0.3

Absorbance of Positive Control (PC)	
Reading-1	2.240
Reading-2	2.184
Average PC reading	2.212

Cutoff value = Av. NC + 0.3 = 0.017 + 0.3 = 0.317

SAMPLE DATA

Well	Absorbance	Mean	Cutoff	Result
NC	0.020	0.017	0.317	
NC	0.014			
PC	2.240	2.212		
PC	2.184			
Sample 1	3.487			Reactive
Sample 2	0.654			Reactive
Sample 3	0.056			Non-Reactive

INTERPRETATION OF RESULTS

1. Samples with absorbance value less than the cut-off value are considered non-reactive by **QUALISA™ HCV** are considered negative for HCV antibodies.
2. Samples with absorbance value equal to or greater than cut-off value are considered reactive by **QUALISA™ HCV.** The original sample should be retested in duplicate. Initially reactive sample that do not react in either of duplicates are considered negative for antibodies to HCV. Initially reactive sample that reacts in either or both duplicates are considered repeatedly reactive.
3. If a sample is repeatedly reactive the probability of antibodies to HCV are high, especially with patients at high risk or high absorbance values. Such samples should be retested with supplemental tests such as RIBA or HCV RNA assay.
4. In case of samples with high OD, there are possibilities of black precipitate formation after the addition of stop solution.This will not interfere with the interpretation of results.

NOTE

All ELISA readers have a linear measuring range (approx. 2.5A°). Beyond this range the OD values are non-linear. That is why some instruments can be pre-programmed to show "out of range" values if the OD exceeds 2.5 or 3.00. **QUALISA™ HCV** positive control may show "out of range" values in such instruments even after dilution. Please note that this indicate a valid run (provided the negative control value is < 0.1) and does not interfere with assay results.

PERFORMANCE CHARACTERISTICS

Two hundred and fifteen samples – out of which fifty positive and one hundred and sixty five negative samples were tested with **QUALISA™ HCV** and compared with a commercially available ELISA kit. The results are given below:

SPECIMEN DATA	TOTAL	QUALISA™ HCV	Commercially available ELISA
Number of specimen tested	215	215	215
Number of Positive specimens	50	50	50
Number of Negative specimens	165	165	165

Based on this evaluation:
Sensitivity of **QUALISA™ HCV** : 100%
Specificity of **QUALISA™ HCV** : 100%

Evaluation with Seroconversion Panel

Results of Seroconversion Panel obtained from Boston Biomedica Inc. (PHV901) are given below:

Panel ID	Days since First bleed	Abbott HCV 2.0	Abbott HCV 3.0	Ortho RIBA 2.0	Ortho RIBA 3.0	Chiron HCV RNA	QUALISA™ HCV
PHV 901-01	0	0.2	0.2	NEGATIVE	NEGATIVE	NEGATIVE	0.25
PHV 901-02	72	0.2	0.2	NEGATIVE	NEGATIVE	NEGATIVE	0.36
PHV 901-03	104	1.6	1.0	POSITIVE	POSITIVE	POSITIVE	4.09
PHV 901-04	106	1.7	1.0	POSITIVE	POSITIVE	POSITIVE	3.22
PHV 901-05	111	1.7	1.2	POSITIVE	POSITIVE	POSITIVE	3.41
PHV 901-06	113	1.6	1.3	POSITIVE	POSITIVE	POSITIVE	3.25
PHV 901-07	138	3.8	9.0	POSITIVE	POSITIVE	POSITIVE	8.96
PHV 901-08	146	3.6	6.8	POSITIVE	POSITIVE	POSITIVE	4.45
PHV 901-09	166	5.0	>10.6	POSITIVE	POSITIVE	POSITIVE	>14
PHV 901-10	173	5.0	>10.6	POSITIVE	POSITIVE	POSITIVE	>14
PHV 901-11	209	5.0	>10.6	POSITIVE	POSITIVE	POSITIVE	>14

Note: All numerical values are expressed as specimen absorbance to cut off ratios. Ratios more than or equal to 1.0 are considered positive. The data other than that for **QUALISA™ HCV** was supplied by BBI.

Precision
Inter & Intra-assay precision studies indicate that the CV is 10 ± 3%.

REMARKS

(1). Approximately 25-30% of individuals with chronic HCV infections have persistently normal alanine aminotransferase (ALT or SGPT) level and these individuals are usually referred to as "healthy carrier" of HCV. However, several studies have demonstrated that the histological features of most healthy carriers showed chronic liver damage of a variable degree, ranging from mild hepatitis to liver cirrhosis, and thus the existence of the true "healthy carrier" of HCV is still debatable. (2). At least six major genotypes of HCV, each comprising multiple subtypes, have been identified worldwide. Apart from genotypes 1 through 6, HCV genotypes 7, 8 and 9 have been identified only in Vietnamese patients and genotypes 10 and 11 were identified in patients from Indonesia. There has been disagreement about the number of genotypes into which HCV isolates should be classified. Investigators have proposed that genotypes 7 through 11 should be regarded as variants of the same group and classified as a single genotype, type 6. (3). Though **QUALISA™ HCV** is a reliable screening assay, it should not be used as a sole criterion for diagnosis of HCV infection.(4). Absence of antibodies to HCV does not indicate that an individual is absolutely free of HCV infection as the collection of sample and its timing vis-a-vis seroconversion will influence the test outcome.(5).Since various tests for HCV differ in their performance characteristics and antigenic composition, their reactivity patterns may differ. (6).Testing of pooled samples is not recommended. (7). As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated. (8). According to CDC, among immuno competent populations with anti-HCV prevalence <10%, the proportion of false-positive results in HCV EIAs averages approximately 35% and among immuno compromised populations, the proportion of false-positive results average 15%. **QUALISA™ HCV** should only be used as a screening test and its results should be confirmed by other supplemental assays like RIBA and NAT.

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