TOYO®

INSTRUCTION FOR USE
Anti-HCV TEST, WB/S/P

Anti-HCV (HCVab) Detection in
Whole Blood / Serum / Plasma

in vitro diagnostic test

Only for professional *in vitro* diagnostic use

Product Code: THC02
Hepatitis C Virus Antibody Cassette Test

BACKGROUND INFORMATION

Hepatitis C virus (HCV) is a major cause of chronic liver disease, frequently progressing to cirrhosis and increased risk of hepatocellular carcinoma. HCV is a positive, single-stranded RNA virus in the Flaviviridae family. The genome is approximately 10.000 nucleotides and encodes a single polyprotein of about 3.000 amino acids. The polyprotein is processed by host cell and viral proteases into three major structural proteins and several non-structural proteins necessary for viral replication. Several different genotypes of HCV with slightly different genomic sequences have since been identified that correlate with differences in response to treatment with interferon alpha.
Recently, new antiviral drugs have been developed. These medicines, called direct antiviral agents (DAA) are much more effective, safer and better-tolerated than the older therapies.
HCV can be classified into six genetically distinct genotypes and further subdivided into at least 70 subtypes, which differ by approximately 30% and 15% at the nucleotide level, respectively. The different genotypes may exhibit differing phenotypic properties. Immunochromatographic membrane tests can be performed in a few minutes and the results are read visually and could be suitable for use in laboratories that have limited facilities. In addition, even if there is no prophylactic HCV treatment after a needle-stick injury, it can be important to know rapidly the HCV status of a source patient.

INTENDED USE

Anti-HCV Test is a rapid chromatographic immunoassay for the qualitative detection of antibodies generated against proteins that are encoded by conserved sequences of core, NS3, NS4, NS5 parts of HCV genome in human whole blood / serum / plasma.

REAGENTS

Recombinant HCV antigens (CORE, NS3, NS4, NS5), anti-HCV monoclonal antibodies, recombinant HCV antigens (CORE, NS3, NS4, NS5) conjugated with colloidal gold particles.

METHOD

Anti-HCV Test uses solid-phase immunochromatographic technology for the qualitative detection of antibodies against HCV antigens in human whole blood / serum / plasma. The test is a two-site immunometric assay in which a combination of monoclonal antibodies and recombinant HCV antigens (CORE, NS3, NS4, NS5) are used to selectively detect antibodies against HCV antigens in samples with a high degree of sensitivity. Recombinant HCV antigens including core, NS3, NS4 and NS5 were immobilized on the test area "T" and anti-HCV monoclonal antibodies were immobilized on the control area "C" of the nitrocellulose membrane. Recombinant HCV antigens including core, NS3, NS4 and NS5 were conjugated with colloidal gold were dried on a conjugate pad. Sample is introduced from sampling pad. If there is anti-HCV in the sample, anti-HCV binds to the mobile recombinant HCV antigens conjugated with colloidal gold particles. Together they move to the test area "T". Anti-HCV - recombinant HCV antigen complex binds to the immobilized recombinant HCV antigens and as a result of this, anti-HCV that have already bound to mobile recombinant HCV antigens (conjugated with colloidal gold particles) become immobilized in the test area "T" thus creating a visible colored signal due to the accumulation of colloidal gold particles in the test area "T" (a colored test line), indicating positive test result. If there is no anti-HCV in the sample then sample moves to the test area "T" together with unbound (free) recombinant HCV antigens conjugated with colloidal gold particles. Immobilized recombinant HCV antigens can not bind to mobilized recombinant HCV antigens conjugated with colloidal gold particles, therefore no visible colored signal in test area "T" (no colored test line) can be obtained, indicating negative test result. Regardless of anti-HCV content of the liquid sample, mobile recombinant HCV antigens conjugated with colloidal gold particles, bind to immobilized anti-HCV monoclonal antibodies while liquid sample is passing through the control area "C". Therefore accumulation of colloidal gold particles produces a visible colored signal in the control area "C" (a colored control line), indicating a valid test result. Colored line should be visible in the control area "C" in every case; if no visible colored line in control area "C", test result should be indicated as invalid.

PRECAUTIONS AND LIMITATIONS

1. For professional and *in vitro* diagnostic use only.
2. Do not use test kit beyond expiry date. The test device is single use. Do not reuse.
3. The test device should remain in its original sealed pouch until usage. Do not use the test if the seal is broken or the pouch is damaged.
4. Wear disposable gloves while performing the test.
5. Use a new dropper for each sample. Plastic dropper supplied with test kit may not drop exact sample volume thus micropipette should be used.
6. The test device should be discarded in a proper biohazard container after testing.
7. All patient samples should be handled as taking capable of transmitting disease into consideration. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of samples.
8. Do not freeze and thaw the serum, plasma samples repeatedly. Using of frozen and thawed samples should be avoided whenever possible, due to the blocking of the membrane by the debris.
9. Do not use turbid, hemolyzed samples. Turbid test samples should be centrifuged.
10. Hemolytic samples should not be used since they can lead to invalid or false results.
11. This test will indicate only the presence or absence of anti-HCV in the sample, and should not be used as the only basis for the diagnosis of hepatitis viral infection.

As with all diagnostic tests, it should be kept in mind that an identification diagnosis can't be based on a single test result.Diagnosis can only be reached by an expert after the evaluation of all clinical and laboratory findings.

STORAGE

Test device should be kept away from direct sunlight, moisture, heat and radiation sources. Store at 4 - 30°C (39 - 86°F). Do not freeze.
The test in the original packaging retains stable until expiry date at storage conditions. The test device should be used in maximum one hour after the foil is opened.

Kit components : Test cassettes, droppers, diluents and instructions for use.
Additional materials required but not provided : Sample collection tube, centrifuge and timer.
Additional materials recommended but not provided : Micropipettes to deliver mentioned amount of sample in the test procedure, negative and positive control materials.

SAMPLE COLLECTION AND PREPARATION

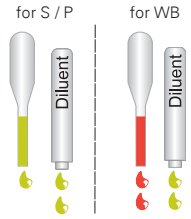
The test can be performed using whole blood, serum or plasma. To avoid hemolysis, serum or plasma should be separated from blood as soon as possible.
For Whole Blood Samples: Test should be performed immediately with whole blood samples. Otherwise, whole blood samples should be stored at 2 - 8 °C with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation until they are being tested in a period of 2 days after collection.
For Serum Samples: Collect blood into a collection tube without anticoagulant, leave to settle for 30 minutes for blood coagulation and then centrifuge the blood. At the end of centrifuge period remaining supernatant is used as serum.(Centrifugation time & speed: 2300-2880 x g for ~ 10 min)
For Plasma Samples: Collect blood into a collection tube with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation of blood sample and then centrifuge the blood. At the end of centrifuge period supernatant is used as plasma.(Centrifugation time & speed: 2300-2880 x g for ~ 10 min)
Do not use turbid, hemolyzed samples. If the sample cannot be tested on the day of collection, store the serum, plasma samples in a refrigerator or freezer. Do not freeze and thaw the serum, plasma samples repeatedly. Do not freeze whole blood sample. Bring the samples to room temperature before testing. Frozen samples must be completely thawed and mixed well prior to testing. Turbid test samples should be centrifuged. Using of frozen and thawed samples should be avoided whenever possible, due to the blocking of the membrane by the debris.

TEST PROCEDURE

1. Bring the tests and whole blood / serum / plasma samples to room temperature, Take the test out of its pouch.
2. **For Whole Blood Samples:** Draw whole blood and put 2 drops (60 μl) into the sample well of the cassette. Immediately after, 2 drops of diluent is added into the sample well and allowed to soak in.

   For Serum / Plasma Samples: Draw serum / plasma and put 1 drop (30 μl) into the sample well of the cassette. Immediately after, 2 drops of diluent is added into the sample well and allowed to soak in. Avoid the formation of any air bubbles.
3. Depending on the anti-HCV concentration in the sample, the test can react even in 5 minutes. Results should be read at 15 minutes as shown below. Do not interpret results beyond 20 minutes, results forming after 20 minutes should be regarded as invalid.

* Micropipette usage is highly recommended *



INTERPRETATION OF RESULTS

Negative: Only one colored line is visible in "C" area, indicating that hepatitis C antibody does not exist.
Positive: Two colored lines are visible in "C" and "T" areas, indicating that hepatitis C antibody exists.
Low concentration of hepatitis C antibody may cause a faint line in "T" area. Even such a faint line in "T" area should be regarded as "positive".
Invalid: No colored line is visible or only one colored line is visible in "T" area; test should be repeated using a new test device.
Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

Tests have built in procedural quality control features. When the test is complete, the user will see a colored line in the "C" area of the test on negative samples and a colored line in the "T" and "C" area on positive samples. The appearance of the control "C" line is considered as an internal procedural control. This line indicates that sufficient volume of sample was added as well as valid test result. It is recommended that a negative control and a positive control be used to verify proper test performance as an external control. Users should follow appropriate federal, state and local guidelines concerning the external quality controls.

PERFORMANCE EVALUATION

Anti-HCV Test can detect antibodies generated against proteins that are encoded by conserved sequences of CORE, NS3, NS4, NS5 parts of HCV genome.

Sample Status	Sample Anti-HCV Status	S / P Sample Type			WB Sample Type		
		Study Number	Com. Assay	Result	Study Number	Com. Assay	Result
Positive samples (all available genotypes)	Positive	400	EIA	100 %	413	EIA	100 %
Blood donors	Negative	1058	EIA	100 %	1011	EIA	100 %
Clinical	Negative	250	EIA	100 %	210	EIA	100 %
Pregnant women	Negative	229	EIA	100 %	212	EIA	100 %
Rheumatoid Factor (RF)	Negative	150	EIA	100 %	150	EIA	100 %
Anti-HBs	Negative	20	EIA	100 %	10	EIA	100 %
Bilirubin	Negative	10	EIA	100 %	-	-	-
Hemoglobin	Negative	10	EIA	100 %	-	-	-
Triglycerides	Negative	10	EIA	100 %	-	-	-

Sensitivity and Specificity

For S/P samples; results of positive samples (400/400), Negative blood donors samples (1058/1058) and clinical samples (250/250) are used. Using these results; sensitivity, specificity, +predictive, - predictive values are calculated as;

Sensitivity: 100 % Specificity: 100 % + Predictive V: 100 % - Predictive V: 100 %

For WB samples; results of positive samples (413/413), Negative blood donors samples (1011/1011) and clinical samples (210/210) are used. Using these results; sensitivity, specificity, +predictive, - predictive values are calculated as;

Sensitivity: 100 % Specificity: 100 % + Predictive V: 100 % - Predictive V: 100 %

Seroconversion panels: 30 seroconversion panels for Türklab Anti-HCV Test were conducted where EIA was the reference assay. All 30 seroconversions were properly detected by the Türklab Anti-HCV Test which reacted positive with the appearance of a line at "C" area for the control and with another line at "T" area for the test sample. The predicate EIA assay also detected the seroconversion of all 30 panels. This evaluation indicates that Türklab Anti-HCV Test has demonstrated an equivalent performance in the diagnostic sensitivity compared to the CE-marked EIA assay.

Cross Reactivity: Cross reactivity has been tested with below samples, no cross reactivity was found with the Anti-HCV Test.
- Anti-HBs whole blood / serum / plasma samples and Anti-HBs positive control.
- Whole blood / serum / plasma samples from pregnant women.

Interferences: Following potentially interfering substances were tested with Anti-HCV Test: Hemoglobin, Bilirubin, Triglycerides, Rheumatoid Factor (RF). No interference was observed.

Hemolytic samples should not be used since they can lead to invalid or false results.

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CE 1434

Manufacturer
Consult instruction for use
Attention, see instruction for use
IVD *In vitro* diagnostic medical device
For single use only
Number of test
REF Catalog number
Storage temperature
LOT Lot number
Expiry date