IVD

ANTIBODY TO HEPATITIS C VIRUS (ANTI-HCV) ELISA KIT

Catalog No: E0320

INTENDED USE

The Autobio anti-HCV assay is designed for the qualitative determination of antibody to Hepatitis C Virus (anti-HCV) concentration in human serum or human plasma.

INTRODUCTION

Hepatitis C virus (HCV), which was formerly described as the parenterally transmitted form of non-A, non-B hepatitis (NANBH) [1], becomes a chronic disease in 50% of the cases[2].

HCV can also be transmitted through intravenous drug abuse, sexual, and household contact.[3]

Hepatitis C virus is a single stranded RNA virus with some structural relations to the flavivirus family. Nucleic acid sequences of HCV cDNA clones provided the basis for the construction of recombinant peptides representing putative hepatitis C virus proteins. Anti-hepatitis C virus antibody screening of blood using synthetic or recombinant proteins[4,5] helped to identify apparently healthy blood donors with anti-HCV antibodies who otherwise might have transmitted the virus. This is an enzyme linked immunosorbent assay using recombinant proteins derived from core regions of HCV virus to detect the presence of HCV antibodies in human sera.

PRINCIPLE OF THE TEST

Multiple epitopes of HCV proteins are bound to the microtiter plate. When antibodies to HCV are present in the test sample, they react with recombinant proteins and attach to the solid-phase. Non-reactive antibodies are removed with the wash buffer. Human IgGs bound to the antigen are reacted with mouse anti-human IgG peroxidase conjugate and visualized by subsequent reactions with a chromogenic substrate. Positive sample generates a medium to dark blue color. No color or very pale blue color indicates a negative reaction. The intensity of the reaction is photometrically quantitated.

MATERIALS PROVIDED

1. Antigen Coated Microtiter Plate: Microplate with HCV recombinant protein coated wells (1 plate, 96 wells).
2. Enzyme Conjugate Reagent: Horseradish Peroxidase (HRP) labelled mouse anti-human IgG in Stabilizing Buffer (1 vial, 11.5ml).
3. Negative Control: Heat inactivated healthy human sera diluted in sample diluent (1 vial, 1.0ml).
4. Positive Control: Heat inactivated human sera containing anti-HCV antibodies diluted in sample diluent (1 vial, 1.0ml).
5. Sample Diluent: Chemically defined solution containing proteins and sodium azide in phosphate buffer (1 vial, 11.5ml).
6. Wash Solution Concentrate: PBS-Tween (1 bottle, 50ml, 20X)
7. Substrate Solution: Hydrogen Peroxide (1 vial, 7.5ml)
8. Chromogen Solution: Tetramethylbenzidine (1 vial, 7.5ml)
9. Stop Solution: 1.0M H_2SO_4 (1 vial, 7.5ml)

MATERIALS NOT PROVIDED

The following materials are required but not provided in the kit:

1. Distilled water
2. Precision pipettes for delivery of 20-200 l, 100-1000 l (the use of accurate pipettes with disposable plastic tips is recommended)
3. Microplate ELISA reader with a bandwidth of 10nm or less and an absorbance range of 0-3.5 or greater at 450nm wavelength
4. Magnetic stirrer
5. Vortex Mixer or equivalent
6. Washer for microplates
7. Quality control specimens
8. Incubator
9. Absorbent paper

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2-8℃ upon receipt and the microplate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (1 year from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable for at least two months, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARATION

1. Plasma samples may be used for this assay but serum is the recommended sample type for this assay.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Allow samples to clot for 1 hour before centrifugation.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Prior to use, specimens should be capped and may be stored up to 48 hours at 2-8℃. For longer storage, freeze the specimens at -20℃. Thawed samples must be mixed prior to testing.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. The control sera contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, the control sera and components containing animal substances should still be treated as potentially infectious.
4. Avoid any skin contact with all reagents. Stop Solution contains H_2SO_4, in case of contact, wash thoroughly with water.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25℃) before use.
2. Adjust the incubator to 37℃.
3. Prepare Wash Solution: add 50ml of Wash Solution Concentrate to 1000ml of distilled water, and mix well with magnetic stirrer. The Wash Solution is stable at room temperature for two months.

IMPORTANT NOTES

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. It is recommended that no more than 32 wells be used for each assay run, if manual pipette is used, since pipetting of all specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipette is available.
4. Replace caps on reagents immediately. Do not switch caps.
5. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Prepare data sheet with sample identification.
2. Write down the relative numbers of specimens and wells on the data sheet. Leave 1 well for the blank, 5 additional wells for the controls and 1 well for each specimen. If the experiment does not need the whole plate, remove the remaining strips from the strip holder and store the unused strips in the ziplock bag (included in the kit) which contains desiccant.
3. Reserve 1 well for blank, add 100 l of the Negative Control to each of 3 wells, 100 l of the Positive Control to each of 2 wells and 100 l of sample diluent to each of the other wells, then pipette 10 l of sample to sample diluent wells.

   Notes: use an individual tip for each pipetting to avoid cross contaminations.
4. Thoroughly mix for 30 seconds. It is important to have complete mixing in this step.
5. Incubate the plate at 37°C for 30 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter plate 6 times with wash solution.

8. Strike the plate sharply onto absorbent paper to remove residual water droplets.
9. Add 100 l of Enzyme Conjugate Reagent to each well (except the blank well). Mix it gently by swirling the microtiter plate on flat bench.
10. Incubate the plate at 37°C for 30 minutes.
11. Repeat steps 6 to 8.
12. Dispense 50μl of the Chromogen Solution into each well.
13. Dispense 50μl of the Substrate Solution into each well. Gently mix for 15 seconds.
14. Incubate the plate at 37°C in the dark for 10 minutes without shaking.
15. Stop the reaction by adding 50μl of the Stop Solution to each well.
16. Gently mix for 15 seconds. It is very important to make sure that the blue color changes to yellow completely.
17. Read absorbance at 450nm with a Microplate ELISA reader within 20 minutes.
18. Record the absorbance results on a data sheet, including the kit's lot number, date, operator and any notes about the run. If a printed copy of the absorbance reading is available, it should be attached to the data sheet.

CALCULATION OF RESULTS

Test is valid if:

1. Mean absorbance of negative control is lower than 0.10.
2. Mean absorbance of positive control is equal to or higher than 0.6.
3. Cut-off value = 0.1+NCx (Mean absorbance of Negative Control)
   If the absorbance value of the negative control is less than 0.05, it should be recorded as 0.05. If it is more than 0.05, it should be recorded as the actual absorbance value measured.

EXAMPLE OF RESULTS

Example: NCx = 0.032

Cut-offvalue = 0.1+ 0.050= 0.150

Any sample, of which the absorbance is equal to or higher than the cut-off value, is considered positive of HCV. Any sample, of which the absorbance is lower than the calculated cut-off value, is considered negative of HCV.

PERFORMANCE

*A. S*ensitivity and *Specificity*

The evaluation of this kit.

The serum wheel contains 500 serum/plasma specimens obtained from (1) 100 specimens from reference laboratories including 67 positive sepcimens and 33 negative specimens. (2) 64 national references of anti-HCV (purchased from China's Natioinal Institute for the Control of Pharmaceutical and Biological Products) including 30 positive references, 30 negative references and 4 sensitivity references. (3) 336 clinical specimens which are collected by reference laboratories from blood donors.

The calculation of the sensitivity, specificity and efficiency

sensitivity = positive/(postive + false negative) × 100%

specificity = negative/(negative + false positive) ×100%

efficiency = (positive + negative)/(positive + false positive + negative + false negative) × 100%

Item	Positive	False Positive	Negative	False Negative	Sensitivity (%)	Specificity (%)	Efficiency (%)
Result	100	2	397	0	100	99.50	99.60

B. Precision

a. Intra-Assay Precision

Intra-Assay Precision was determined by assaying each of the 2 control sera in 20 replicates.

Serum	**Number**	**Mean**	**SD**	**RSD（%）**
High	20	1.056	0.058	5.4%
Low	20	0.323	0.024	7.4%

b. Inter-Assay Precision

Inter-assay Precision was determined by assaying in duplicates 2 serum pools in 20 separate runs.

Serum	**Number**	**Mean**	**SD**	**RSD（%）**
High	20	1.099	0.077	7.0%
Low	20	0.334	0.031	9.2%

LIMITATIONS

1. As the other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. So do aspirate the well or get rid of entire content of wells completely before adding the washing solution.
2. Aswith all diagnostic tests, a definitive clinical diagnosis should not be made based only on the results of a single test. A complete evaluation by physician is needed for a final diagnosis.
3. The test is for research use, further manufacturing and export only.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

SYMBOLS

LOT	**BATCH CODE**
	**USE BY**
	**MANUFACTURER**
	**CONTAINS SUFFICIENT FOR <n> TESTS**
IVD	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**
	**TEMPERATURE LIMITATION**
REF	**CATALOGUE NUMBER**
	**CONSULT INSTRUCTIONS FOR USE**

REFERENCES:

[1] Kuo,G, Choo Q-L, Alter, HJ, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 1989. 244:362-4.

[2] Esteban JI, Gonzalez A, Hernandez JM, et al. Evaluation of antibodies to hepatitis C virus in a study of transfusion-associated hepatitis. N Engl J Med 1990. 323:1107-12.

[3] Miyamura T, Saito I, Katayama T, et al. Detection of antibody against antigen expressed by molecularly cloned hepatitis C virus cDNA: application to diagnosis and blood screening for posttransfusion hepatitis. Proc Natl Acad Sci USA 1990. 87:983-7.

[4] Estaban JI, Esteban R, Viladomiu L, et al. Hepatitis C virus antibodies among risk groups in Spain. Lancet 1989. 2:294-7.

[5] Houghton M, Weiner A, Han J, Kuo G, Choo Q-L. Molecular Biology of the Hepatitis C viruses: Implications for diagnosis, Development, and Control of Viral Disease. Hepatology 1991. 14:381-8.

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