Anti-HCV

Antibody to Hepatitis C Virus（CLIA）

Order Information

Catalog No.	Package Size
Anti-HCV111	2×50 tests
Anti-HCV112	2×100 tests

Intended Use

The CL-series Anti-HCV assay is a Chemiluminescent Immunoassay (CLIA) for the qualitative determination of antibody to hepatitis C virus (Anti-HCV) in human serum or plasma.

Summary

Hepatitis C virus (HCV) is an enveloped, positive-sense single-stranded RNA virus which has been classified as an own genus in the family of Flaviviridae. It is the most common cause of bloodborne[1-3] as well as community-acquired non-A, non-B hepatitis worldwide[4]. The HCV genome consists of ~9.5 kb encoding for a 3000 amino acid polypeptide of structural and non-structural domains[5]. Like other RNA viruses, and exhibits substantial heterogeneity as a result of mutations that occur during viral replication. The presence of anti-HCV indicates that an individual may have been infected with HCV, may harbor infectious HCV, and/or may be capable of transmitting HCV infection[6]. Anti-HCV antibody tests are used alone or in combination with other tests (e.g. HCV-RNA) to detect an infection with hepatitis C virus and to identify blood and blood products of individuals infected with HCV.

Assay Principles

The CL-series Ant-HCV assay is a two-step sandwich assay to determine the level of antibody to hepatitis C virus.

In the first step, sample, sample treatment solution, paramagnetic microparticles coated with recombinant HCV antigens are added into a reaction cuvette. After incubation, antibodies to HCV present in the sample bind to HCV antigens coated on microparticles. Afterwards, microparticles are magnetically captured while other unbound substances are removed by washing.

In the second step, diluent solution and alkaline phosphatase labeled anti-human IgG monoclonal antibody conjugate are added into the reaction cuvette. After incubation, anti-human IgG monoclonal antibody conjugate binds to anti-HCV antibodies captured on microparticles. Microparticles are magnetically captured while other unbound substances are removed by washing.

In the third step, the substrate solution is added into the reaction cuvette. It is catalyzed by alkaline phosphatase in the immune complex captured on the microparticles. The resulting chemiluminescent reaction is measured as relative light units (RLUs) by a photomultiplier built inside the system. A direct relationship exists between the amount of anti-HCV antibodies in the sample and the RLUs generated during the reaction.

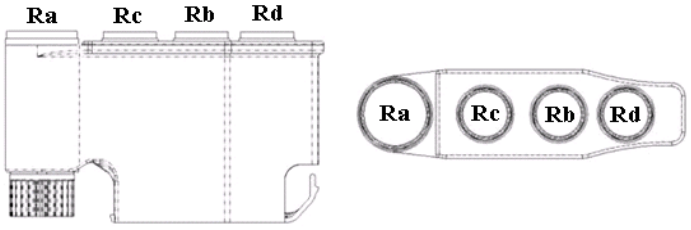
The presence or absence of anti-HCV antibodies in the sample is determined by comparing the chemiluminescent signal of the reaction to the cutoff signal determined from system calibration. A cutoff index (COI) is calculated by Sample RLU/Cutoff RLU.

Reagents Components

The reagent kit is composed of four components: Ra, Rb, Rc, and Rd. The component cannot be exchanged, and the detailed information of each component is listed below：

Ra	Paramagnetic microparticles coated with recombinant HCV antigens in MES buffer with preservative.
Rb	Alkaline phosphatase labeled anti-human IgG monoclonal antibody conjugate in MES buffer with preservative.
Rc	Sample diluents in HEPES buffer with preservative.
Rd	MES buffer with preservative.

The position of each component is shown in the figure below (front view on the left and top view on the right):



Storage Stability

The unopened Anti-HCV (CLIA) reagent is stable up to one full year when stored at 2-8 ^{0}C, the actual expiration date is stated on the label.

The Anti-HCV (CLIA) reagent can be stored onboard at 2-8 ^{0}C and used for a maximum of 28 days after opening

for use.

Reagent Preparation

Ra: Ready to use
Rb: Ready to use
Rc: Ready to use
Rd: Ready to use

Materials required but not provided

Mindray CL-series Chemiluminescence Immunoassay Analyzer.
Cat. No. Anti-HCV211: Anti-HCV Calibrators, 1×2.0mL for each level of calibrator C0 and C1.
Cat. No. Anti-HCVN311: Anti-HCV Negative Control 3×2.0mL.
Cat. No. Anti-HCVN312: Anti-HCV Negative Control, 6×2.0mL.
Cat. No. Anti-HCVP311: Anti-HCV Positive Control, 3×2.0mL.
Cat. No. Anti-HCVP312: Anti-HCV Positive Control, 6×2.0mL.
Cat. No. CS511: Substrate Solution, 4×115mL.
Cat. No. WB411: Wash Buffer.
Reaction Vessel.

Instrument System

Mindray CL-series Chemiluminescence Immunoassay Analyzer.

Specimen Collection and Preparation

Human serum and plasma collected sodium heparin and lithium heparin are recommended for this assay.
Collect all blood samples observing routine precautions for venipuncture. Follow blood collection tube manufacturer's recommendations for centrifugation. Centrifuge the specimens after clot formation is complete. Some specimens, especially those from patients receiving anticoagulant therapy may exhibit increased clotting time. Please ensure that residual fibrin and cellular matter have been removed prior to analysis.
For optimal results, inspect all samples for bubbles. Remove bubbles with a pipette tip prior to analysis. Specimens must be mixed thoroughly after thawing. Thawed samples should be centrifuged prior to use. If the sample was covered with lipid layer after centrifugation, the sample should be transferred to a clean tube before testing. Do not transfer the lipid layer. Handle carefully to prevent cross contamination. Do not use grossly hemolyzed specimens. Do not use heat-inactivated specimens.
Specimens should be tested as soon as possible after sample collection. If testing is not completed within 8 hours, specimens should be stored at 2-8°C or colder. Specimens would be stable for 7 days at 2-8°C, 3 months at -20°C. Avoid more than five freeze cycles.

Assay Procedure

For optimal performance of this assay, operators should read the related system operation manual carefully to get sufficient information, such as operation instructions, sample preservation and management, safety precautions, and maintenance. Prepare all required materials for the assay as well.
Before loading the Anti-HCV reagent kit on the instrument for the first time, invert unopened reagent bottle gently for at least 30 times to resuspend the microparticles, which have settled during shipment or storage. Visually inspect the bottle to ensure the microparticles have been resuspended. If the microparticles remain adhered to the bottle, continue inverting until the microparticles have been completely resuspended. If the microparticles cannot be homogenized, it is recommended not to use this bottle of reagent. Contact Mindray Customer Service for help. Do not invert opened reagent bottle.
This assay requires 20 μL of sample volume for a single test. This volume does not include the dead volume of the sample container. Additional volume is required when performing additional tests from the same sample. Operators should refer to the system operation manual and specific requirement of the assay to determine the minimum sample volume.

Calibration

The calibration information is stored in the barcode attached in the reagent and calibrator pack. When performing the calibration, scan the information from the barcodes into the system first, and then test the calibrators at two levels. Calibration is required before any Anti-HCV test. Recalibration is recommended every 4 weeks, or when a new reagent lot is used, or the quality controls are out of specified range. For detailed instruction of calibration, refer to the system operation manual.

Quality Control

For verifying the reliability of assay system, quality

controls should be run at least once every 24 hours, after a new reagent pack is loaded, or after every calibration. The recommended quality controls for this assay are Mindray Anti-HCV Negative Control and Anti-HCV Positive Control.

Quality control results should be within the acceptable ranges. If a control is out of its specified range, the associated test results are invalid and the samples must be retested. Recalibration may be required. Refer to the system operation manual to check up the system. If the quality control results are still out of the specified ranges, please contact Mindray Customer Service for help.

Calculation

The CL-series analyzer automatically calculates Cutoff RLU using the mean RLU from three replicates of calibrator C0 and two replicates of calibrator C1, and stores the results. Then the analyzer calculates Anti-HCV result based on the cutoff RLU by the following calculation.

Cutoff RLU = [(Mean RLU of C1 – Mean RLU of C0) x Calibration Coefficient] + Mean RLU of C0.

Calibration Coefficient is specific for each combination of reagent kit lot and calibrator lot.

COI = Sample RLU/Cutoff RLU.

Interpretation of Results

Samples with a COI<1.00 are Non-reactive in the Mindray Anti-HCV assay. These samples do not need further testing.

Samples with a COI≥1.00 are Initially Reactive in the Mindray Anti-HCV assay. All Initially Reactive samples should be transferred to a centrifuge tube and centrifuged at≥10,000 RCF (Relative Centrifugal Force) for 10 minutes, and retested in duplicate. If COI<1.00 in both retests, the sample is considered as Non-reactive for Anti-HCV. If COI>1.00 in either of the retests, the sample is Repeatedly Reactive for Anti-HCV.

Repeatedly reactive samples should be investigated by supplemental tests such as other HCV specific immunoassays and immunoblot assays or a combination thereof and/or NAT tests.

Limitation of Measurement

The Anti-HCV result of a given specimen can vary, in assays from different manufacturers, due to differences in assay methods, calibration, and reagent specificity. The assay results should be used in conjunction with other data, such as symptoms, results of other tests, and clinical history, etc. to make clinical decisions.

Performance Characteristics

Sensitivity

400 specimens have been claimed to be anti-HCV positive by reference methods. Mindray Anti- HCV assay on CL-2000i system has detected 399 samples as positive and 1 samples as negative. The positive coincidence rate is 99.75%.

Precision

The CL-series Anti-HCV assay is designed to have a precision of ≤10% (within-device CV). Precision was determined by following National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP5-A2[7]. Two levels of quality controls were tested in duplicate in two separate runs per day, for a total of 20 days, using a single lot of reagents and a single calibration curve. The precision data are summarized in the table below.

Sample	Mean Anti-HCV (COI)	Within-Run CV	Between-Run CV	Within-Device CV
Negative Control	0.21	2.09%	5.34%	8.09%
Positive Control	3.93	2.48%	1.69%	3.34%

Specificity

Hemoglobin up to 500 mg/dL, bilirubin up to 20 mg/dL, triglycerides up to 3000 mg/dL, and total protein up to 10 g/dL will not interfere with the CL-series Anti-TP assay. With these substances at indicated concentration , the difference is < 0.1 COI on negative specimens and ＜ 10% COI on positive samples .

No obvious interference was observed from rheumatoid factor (up to 1000 IU/mL) or antinuclear antibody.

Mindray Anti-HCV assay was evaluated for potential cross-reactive substances from individuals with medical conditions unrelated to HCV infection. At least 5 samples containing each of the potentially interfering pathogens of the following (HAV, HEV, HBV, CMV, HSV, HIV-1/2, Toxo, RV, TP, EBV, *E.coli*） are tested by Mindray Anti-HCV and a reference reagent kit. All results are consistent between Mindray assay and the reference assay.

623 specimens have been claimed as Anti-HCV negative by reference methods. Mindray Anti-HCV assay on CL-2000i system detected 621 samples as negative and 2 samples as positive. The negative coincidence rate is 99.68%.

Warnings and Precautions

1. For *in vitro* diagnostic use only.

2. Follow all the rules in handling laboratory reagents and take necessary safety precautions.

3. Due to the differences in methodology and antibody specificity, test results of the same sample may be different when using reagent kits from different manufacturers on Mindray system, or using Mindray reagent kits on other systems.

4. Do not use reagent kits beyond the expiration date.

5. Do not use reagents mixed from different reagent lots.

6. Always keep the reagent pack in the upright position to ensure no microparticle has been lost prior to use.

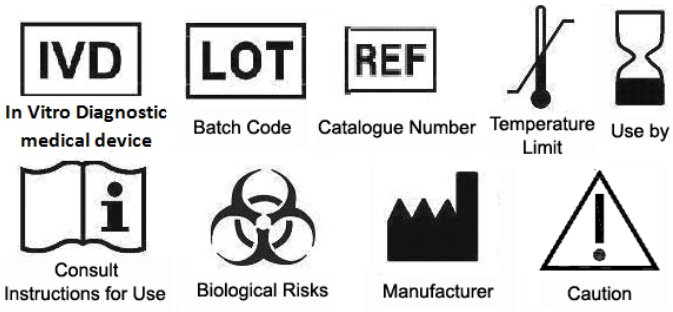
7. Reagent pack opened for more than 28 days is not recommended for use.

8. Reliability of assay results cannot be guaranteed if the instructions in this package insert are not followed.

9. All the specimen and reaction wastes should be considered potentially biohazard. Specimens and reaction wastes should be handled in accordance with the local regulations and guidelines.

10. The Material Safety Data Sheet (MSDS) is available upon request.

Graphical Symbols



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