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Immunoassay

REF **CMC0601/ CMC0602/ CMC0603/ CMC0604/ CMC0605**

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

Anti-HCV CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the qualitative detection of Anti-HCV (IgG antibodies to hepatitis C virus) in human serum and plasma and used as a screening assay.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
			date of manufacture

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IVD

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Introduction

Hepatitis C virus (HCV) is the most frequent cause of parentally transmitted and sporadic chronic non-A, non-B hepatitis (NANBH) [1.2]. It is a single stranded RNA virus, is a member of the family Flaviviridae[3.4]. Six major genotypes (1-6) and a series of subtypes of HCV have been identified. Genotypes 1-3 show a worldwide distribution while genotypes 4 and 5 appear predominantly in Africa and genotype 6 in Asia[5].

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]. HCV is transmitted primarily by exposure to infected blood. Major risk factors associated are illicit intravenous drug abuse and transfusion of blood products before the establishment of cases without an identifiable risk factors[7.8.9]. However, prevalence of HCV infection and associated risk factors vary by geographic region[7]. Estimates of HCV prevalence in blood donors world-wide range is from 0.5 to 8%[10]. HCV virus is present in blood 2–14 days after initial exposure, HCV-specific antibodies are produced 20–150 days after exposure[11]. Although the majority of infected individuals may be asymptomatic, HCV infection may develop into chronic hepatitis, cirrhosis, and/or increased risk of hepatocellular carcinoma[12].

Diagnosis of HCV is dependent on the direct detection of viral RNA by PCR or by detection of Anti-HCV antibodies. Recombinant DNA techniques have been used to develop structural and non-structural proteins derived from HCV RNA with utility for antibody screening. Evolution of screening assays for detection of Anti-HCV antibodies has resulted in generation of much more sensitive test. Anti-HCV CLIA Microparticles has been designed to detect antibodies to putative structural and nonstructural proteins of the HCV genome.

Measurement Principle

This assay is based upon the two-step indirect method. The sample, recombinant HCV antigen coated microparticles and Sample Diluent are combined. Anti-HCV present in the sample binds to the HCV antigen coated microparticles. After washing, Enzyme Conjugate is added. During the incubation, a complex is generated among the solid phase, the Anti-HCV within the sample and HRP-conjugated anti-human IgG by immunological reactions. Chemiluminescent Substrate is added and the complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of Anti-HCV in the samples.

Materials provided

1. Positive Control

1 vial (1.0 mL). Human serum or plasma containing anti-HCV in Tris-HCl buffer with bovine serum. Contains 1‰ ProClin 300® and 1‰ Bronidox preservatives. The Positive Control is tested positive for antibodies to HCV.

Reagent provided ready to use.

2. Negative Control

1 vial (1.0 mL). Tris-HCl buffer contains bovine serum. Contains 1‰ ProClin 300® and 1‰ Bronidox preservatives.

Reagent provided ready to use.

3. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2
Sample Diluent	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2

- Microparticles Solution

HCV recombinant antigens expressed in E.coli coated microparticles in PBS buffer containing BSA. Contains 1‰ ProClin 300® and 0.2‰ Thimerosal preservatives.

- Enzyme Conjugate

Horseradish-peroxidase labeled mouse monoclonal anti-human IgG in PBS buffer and casein. Contains 0.5‰ ProClin 300® and 1‰ Bronidox preservatives.

- Sample Diluent

Anti-HCV Sample Diluent containing Tris-NaCl buffer and casein. Contains 1‰ ProClin 300® and 1‰ Bronidox preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or Autolumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample cup(s) or tube(s) for sample containing
4. Chemiluminescent Substrate ([REF] CMO0101/CMO0102/CMO0103)
5. System Wash for washing the pipetting needle ([REF] CMO0401/ CMO0403)
6. Wash Buffer used in washing procedure ([REF] CMO0301/CMO0302 CMO0303/CMO0304/CMO0305/CMO0306)
7. Distilled or deionized water

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: It is recommended that all materials of human origin be considered potentially infectious. This assay contains materials of animal origin.
6. Some reagents contain ProClin 300® may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately and show this container or label.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
10. Do not use reagents beyond the labeled expiry date.
11. Do not mix or use components from kits with different batch codes.
12. When storing the controls, be certain the vials are securely sealed.
13. Ensure the microparticles are resuspended before loading it on the analyzer.
14. Avoid foam formation in all reagents and sample types (samples and controls).
15. Do not substitute any reagent in this kit from other manufacturers or

other lots.

16. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the remaining Positive or Negative Control to 2-8℃ immediately after the experiment, under which conditions the stability will be retained for 1 month.

Sample

1. The plasma sample types include EDTA, heparin or sodium citrate.
2. Do not use samples with the following conditions:
   - heat-inactivated
   - pooled
   - grossly hemolyzed (>5mg/mL)
   - obvious microbial contamination
   - cadaver samples or any other body fluids
   - sodium azide preservative
3. Collect samples in accordance with correct medical practices. After the blood collection, please follow the tube manufacturer's processing instructions for serum or plasma collection tubes.
4. Ensure complete clot formation in serum samples before centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time.
5. Samples from heparinized patients may be partially coagulated and contain fibrin. Draw the sample prior to heparin therapy.
6. For accurate results, serum and plasma samples should be free of fibrin, red blood cells or other particulate matter.
7. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
8. For optimal results, inspect all samples for bubbles. Remove bubbles with a pipette tip prior to analysis. Use a new tip for each sample to prevent cross contamination.
9. Samples must be separated from clots or red blood cells using centrifugation as recommended by the tube manufacturer. Gravity separation is not sufficient for sample preparation.
10. To ensure consistency in results, samples containing particulate matter or red blood cells, samples that have been thawed, and samples that require retesting must be transferred to a centrifuge tube and centrifuged for 10 minutes prior to testing.
11. Mix thawed samples by inverting 10 times. Visually inspect the samples for the absence of stratification. If layering or stratification is observed repeat inversion cycles until samples are visibly homogeneous. Centrifuge prior to testing.
12. Centrifuged samples with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified sample without the lipemic material.
13. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
14. Samples may be stored at 2-8℃ up to 7 days. Or freeze the samples that need to be stored for more than 7 days at -20℃ or colder. Avoid multiple freeze-thaw cycles, do not freeze-thaw more than 5 cycles.
15. Samples may be shipped at 2-8℃ or -20℃ or colder. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.

Measurement Procedure

1. Check the consumable materials

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample cup(s) or tube(s) on the sample rack, 10 μL of sample for each test. But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution and Sample Diluent to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine Anti-HCV in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibration

- Analyzer can read the bar code on the reagent pack automatically to obtain the essential information for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the Anti-HCV CLIA Microparticles Positive and Negative Controls into the sample cup(s) or tube(s) and place them on the sample rack. They are automatically tested in triplicate or duplicate (triplicate for Positive Controls and duplicate for Negative Controls) at the beginning of each batch. The Assay Analyzer system will not generate results when controls values do not meet specifications. This may indicate either deterioration or contamination of reagents, or analyzer failure.
- Load the sample rack and input Negative & Positive Control information on the system software interface.
- Select "run" to start the test, calibration is required every 28 days.
- Once the control results is accepted and stored, all subsequent

samples may be tested without further calibration unless:
- Controls are out of range after repeated measurements
- A reagent kit and Chemiluminescent Substrate with new batch code is used
- Beyond the expiration date of calibration
- Important parts of the analyzer are replaced or repaired

- Refer to the Assay Analyzer's operation manual.

Measurement Results

- **Calculation**

The Assay Analyzer system calculates the Anti-HCV CLIA Microparticles assay Cut-off value using the following formula:

1. Cut-off Values = Positive Control mean RLU Value x 0.4
2. S/CO = Sample RLU/Cut-off Value
3. Assay analyzer calculates a result based on the samples RLU to the cut-off value for each sample and control.

- **Interpretation of Results**

Samples with S/CO values <1.00 are considered nonreactive (NR).
Samples with S/CO values ≥ 1.00 are considered reactive (R).

Note: All samples that are initially reactive must be centrifuged and retested in duplicate.

All initial reactive samples should be retested in duplicate. If both retest values are nonreactive, the sample must be considered nonreactive for Anti-HCV. If either of the retest values is reactive, the sample must be considered repeat reactive for Anti-HCV by the criteria of this assay.

Repeat reactive Anti-HCV samples should be investigated further in supplemental tests such as other HCV specific immunoassays and immunoblot assays and/or NAT tests.

Although the association of infectivity of donated blood or plasma and the presence of Anti-HCV is strong, it is recognized that presently available methods for Anti-HCV detection are not sensitive enough to detect all potentially infectious units of blood, plasma, or possible cases of HCV infection. A nonreactive test result does not exclude infection.

Control Procedure

The recommended control requirement for this assay involves using positive and negative controls to verify assay performance. The result is valid if the following assigned specification for the controls is met:

Mean PC RLU/ Mean NC RLU >10

When the controls fail to meet assigned specifications, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. It is recommended that each laboratory establish its accepted range to ensure proper test performance.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. False-reactive test results can be expected with any test kit. False-reactive test results have been observed due to nonspecific interactions.
4. Some samples that have undergone multiple freeze-thaw cycles or have been stored frozen for prolonged periods may result in erroneous or inconsistent test results.
5. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
6. This assay was designed and validated for use with human serum and plasma from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
7. Due to limitation of methodology or immunological specificity and other reasons, the test results from different manufacturers' reagents for the same sample may be different, so such results should not be directly compared with each other, so as to avoid the wrong medical explanation. It is recommended that the characteristics of the different manufacturers' reagents should be indicated when reported to the clinician.
8. Patients with impaired immune function or undergoing immunosuppressive therapy, such as HIV patients or patients undergoing immunosuppression after organ transplantation. The reference value of serological antibody testing is limited and may lead to erroneous medical explanations.
9. Some hemodialysis, immune dysfunction and autoimmune diseases patients may be false positive to anti-HCV, thus anti-HCV RIBA or HCV RNA detection can help to confirm and diagnose whether these patients with HCV infection.

Performance Characteristics

Evaluation of performance has been conducted in accordance with the Common Technical Specification (CTS).

1. Measurement Precision

The precision is based on the S/CO from 3 internal controls (1-high, 2-medium and 3-low) tested in replicates of 2 per run with 2 runs per day for 20 days on 3 batches of reagents. Data from this study are summarized in the following table.

Samples	Lot	n	Total	
			Mean	%CV
1	1	80	13.34	6.39
1	2	80	12.94	5.36
1	3	80	13.94	5.76
2	1	80	4.55	8.08
2	2	80	4.40	9.17
2	3	80	4.75	9.04
3	1	80	1.16	7.72
3	2	80	1.16	7.25
3	3	80	1.18	7.41

*Representative data; results in individual laboratories may vary from these data.

2. Sensitivity

In total 342 samples from HCV positive blood donors were tested with the Anti-HCV CLIA Microparticles. All samples obtained positive test results. The sensitivity of Anti-HCV positive samples is 100%.

In total 51 blood donor samples with weak S/CO values on the CE marked commercial assays were tested with the Anti-HCV CLIA Microparticles. All samples obtained positive test results. The sensitivity of Anti-HCV weak positive samples is 100%.

In total 92 genotyped samples from HCV positive blood donors were tested with the Anti-HCV CLIA Microparticles. All samples obtained positive test results.

3. Specificity

In total 5160 negative blood donors from two different European blood donation centers were tested with the Anti-HCV CLIA Microparticles test as well as with the CE marked commercial assay. 8 out of 5160 (5152/5160) samples were tested repeat reactive, which obtained as false positive test results. And they were negative by CE marked

commercial kit and HCV Immunoblots. The specificity of negative blood donors is 99.84%.

252 samples from patients from Frankfurt were screened with the Anti-HCV CLIA Microparticles. One sample was repeat positive but negative by the CE marked commercial assays and confirmed negative with the HCV Immunoblots assay. The specificity of hospitalized patient samples 99.6%.

4. Analytical specificity

Cross reaction: this assay was evaluated for potential cross-reactivity for samples from individuals with medical conditions unrelated to HCV infection. The samples were tested using the Anti-HCV CLIA Microparticles assay. The data are summarized in the following table.

Category	No.	Anti-HCV CLIA Microparticles assay	
		Reactive	Nonreactive
HIV Positive	3	0	3
TP Positive	5	0	5
HBV Positive	20	0	20
HEV Positive	10	0	10
EB VCA-IgA Positive	5	0	5
HAV Positive	5	0	5
Torch Positive	49	0	49
RF Positive	5	0	5
ANA Positive	5	0	5
Pregnant Women	100	0	100

a. Rh.[a] factor: Rheumatoid factor

A total of 207 samples were evaluated, and the results have no cross-reactivity with the test.

Interference: 2 HCV Ab positive samples (P1 and L2) and 2 HCV Ab negative samples (N1 and N2) were spiked with different concentrations of the potentially interfering endogenous substances bilirubin. haemoglobin and triglycerides. All tests were performed in triplicate.

According to the results, at the concentrations listed below, the tested substances have no interference.

- Bilirubin ≤0.4 mg/ mL
- Haemoglobin ≤5mg/mL
- Triglycerides ≤50mg/mL
- Albumin ≤24g/dL

5. Specimen Type

The following specimen types are acceptable for use with this assay:

* Serum
* EDTA-K3, sodium heparin or sodium citrate plasma

Different sample types were taken from 25 non-infected individuals. One part of these samples was tested as such and one part was spiked with an anti-HCV antibody positive sample to a detectable level. The results showed no statistically significant difference between serum and plasma. Anti-HCV CLIA Microparticles assay is suitable for testing serum and plasma samples with anti-coagulants EDTA-K3, sodium citrate and sodium heparin.

6. Seroconversion Panels

A total of 30 commercially available seroconversion panels were tested with the Anti-HCV CLIA Microparticles. The results were compared to the results obtained on 15 CE marked HCV Ab tests. The test performs state of the art for the detection of HCV antibody comparing with other CE marked assays.

Literature References

1. Kuo G, Choo QL, Alter HJ, *et al*. (1989): An assay for circulating antibodies to a major etiology virus of human non-A, non-B hepatitis.

2. Alter MJ, Hadler SC, Judson FN, *et al*. (1989): Risk factors for acute non-A, non-B hepatitis in the United States and association with hepatitis C infection.

3. Miller, R.H. and Purcell, R.H. (1990). Hepatitis C virus shares amino acid sequence similarity with pestiviruses and flaviviruses as well as two plant virus supergroups. Proc Natl Acad Sci 87, 2057.

4. Weiner, A.J., Brauer, M.J.*et al* (1991). Variable and hypervariable domains are found in the regions of HCV corresponding to the flavivirus envelope and NS1 proteins and the pestivirus envelope glycoproteins. Virology180, 842.

5. Centers for Disease Control and Prevention. (2003). Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. MMWR, 52(RR-3): 1-15.

6. Esteban Ji, Gonzalez A, Hernandez JM *et al (*1990). Evaluation of antibodies to hepatitis C virus in a study of transfusion-associated hepatitis *N Engl J Med;* 323:1107-1112.

7. Memom MI, Memom MA *(*2002). Hepatitis C: an epidemiological review. *J Viral Hepat;* 9: 84-100.

8. Conry-Cantilena C, VanRaden M, Gibble J, Melpolder J, Shakil AD, Viladomiu L, Cheung L, *et al (*1996). Routs of infection, Viremia, and liver disease in blood donors found to have hepatitis C virus infection. *N Engl J Med*; 334:1691-1696.

9. Poynard T, Yuen MF, Ratziu V, Lai CL (2003). Viral hepatitis C. Lancet; 362:2095-3000.

10. Conroy-Cantilena, C. (1997). Hepatitis C virus diagnostics: technology, clinical applications and impacts. Trends Biotech 15, 71.

11. Busch MP (2001). Insights into the epidemiology, natural history and pathogenesis of hepatitis C virus infection from studies of infected donors and blood product recipients Transfus Clin Biol. 8: 200–06.

12. Dienstag JL (1983). Non-A, non-B hepatitis. I. Recognition, epidemiology and clinical features. Gastroenterology 85:439-462.