Anti-HCV

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Antibodies to hepatitis C virus (ELISA)

REF: DS177756 Σ 96

Intended use
The DiaSino Anti-HCV assay is an enzyme-linked immunosorbent assay (ELISA) for the qualitative determination of HCV virus in human serum and plasma.

Summary
Hepatitis C virus is the most common cause of posttransfusion and community-acquired non-A, non-B hepatitis worldwide. Infection with HCV frequently leads to chronic hepatitis and cirrhosis, and is associated with the development of hepatocellullar carcinoma.[1]
Hepatitis C virus is an enveloped, positive-sense single-stranded RNA virus which has been classified as an own genus in the family of Flaviviridae. The genome consists of ~9.5 kb encoding for a 3000 amino acid polypeptide of structural and non-structural domains.[2] Like other RNA viruses, the HCV genome exhibits substantial heterogeneity as a result of mutations that occur during viral replication. Worldwide, at least 11 genetically distinct genotypes and multiple subtypes and virus variants have been described.[3] Infection with specific genotypes can affect disease severity and treatment response.[4]
Hepatitis C is primarily transmitted through contaminated blood and blood products and to a lower extent by human body secretions.[5]

Test principle
Indirect method. Total duration of assay: **100 minutes**
The DiaSino Anti-HCV ELISA employs solid phase, indirect ELISA method for detection of antibodies to HCV in two-step incubation procedure. Polystyrene microwell strips are pre-coated with recombinant, highly immunoreactive antigens corresponding to the core and the non-structural regions of HCV (NS3,NS4 and NS5), that can detect all HCV genotypes. During the first incubation step, Anti-HCV specific antibodies, if present, will be bound to the solid phase pre-coated HCV antigens. The wells are washed to remove unbound serum proteins, and mouse anti-human IgG antibodies (anti-IgG) conjugated to the enzyme horseradish peroxidase (HRP-Conjugate) are added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-antibody(IgG) complexes previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells and in presence of the antigen-antibody-anti-IgG (HRP) immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells, and to the amount of antibody in the sample respectively. Wells containing samples negative for Anti-HCV remain colorless.

Reagents
Materials provided
- **Anti-HCV Coated Microplate - symbol** HCV PLATE 8 x 12 strips, 96 wells. Pre-coated with recombianant antigens to HCV.
- **Anti-HCV Negative Control - symbol** HCV NC 1 vial, 0.2 mL, ready to use. Contains 0.1% ProClin300 preservative.
- **Anti-HCV Positive Control - symbol** HCV PC 1 vial, 0.2 mL, ready to use. Contains 0.1% ProClin300 preservative.
- **Anti-HCV Enzyme Conjugate - symbol** HCV CONJ 1 vial, 11.0 mL, ready to use. Contains 0.1% ProClin300 preservative. HRP-Conjugate.
- **Anti-HCV Sample Diluent - symbol** HCV DILUT 1 vial, 11mL.
- **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU:** 1 copy.
- **Plate Lid**: 2 pieces.

Materials required (but not provided)
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 10 µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.
- **Indications of instability deterioration of the reagent:** Values of the Positive or Negative controls, which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one.

Storage and stability
- Store at 2-8℃.
- Don't freeze.
- Seal and return unused reagents to 2-8℃, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation
- Human serum or plasma is recommended for this assay.
- Cap and store the samples at 18-25 ℃ for no more than 8 hours. Stable for 7 days at 2-8℃, and 1 month at -20℃. Freeze only once.
- Do not use heat-inactivated samples.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Avoid grossly hemolytic, lipemic or turbid samples.

Wash solution (40X dilution)
Add deionized water to the 40X concentrated Wash Solution Concentrate.
Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Test procedure
Ensure the patients' samples and reagents are at ambient temperature (18-25 ℃) before measurement. Mix all reagents through gently inverting prior to use.
- **Preparation**: Mark two wells as Negative control (e.g. B1, C1), two wells as Positive control (e.g. D1, E1) and one Blank (e.g. A1, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.
- **Adding Sample Diluent:** Add **100µl** of Sample Diluent into their respective wells except the Blank.
- **Adding Sample**: Add **10 µL** of **Positive control, Negative control,** and **Specimen** into their respective wells except the Blank. Mix by tapping the plate gently. Use a separate disposal pipette tip for each specimen to avoid cross-contamination.
  Note: After adding Sample, the reagents in wells turns **Blue** color from **Green.**
- **Incubating:** Cover the plate with the plate cover and incubate for **60 minutes at 37℃**.
- **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash Buffer. Each time allow the microwells to soak for **30-60 seconds**. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
- **Adding Conjugate:** Add **100 µL** of **Conjugate** into each well except the Blank.
- **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37℃**.
- **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash Buffer. Each time allow the microwells to soak for **30-60 seconds**. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
- **Coloring:** Add **100 µL** of **Substrate** into each well including the Blank. Incubate the plate at room temperature for 10 minutes avoiding light. The enzymatic reaction between the TMB substrate and the HRP-Conjugate produces blue color in Positive control and in Anti-HCV positive sample wells.
- **Stopping:** Using a multichannel pipette or manually, add **50 µL** of **Stop Solution** into each well and mix gently. Intensive yellow color develops in Positive control and Anti-HCV positive sample wells.
- **Measuring:** Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**.

Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within **10 minutes** after stopping the reaction).

Quality control and calculation
Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) value to the Cut-off value (C.O.) of

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the plate. The results should be calculated by subtracting the blank well A value from the print report values of specimens and controls.

Calculation of the Cut-off value (C.O.) = NC + 0.10 (**NC** = the mean absorbance value for two negative controls).
Important: If the NC is lower than 0.05, take it as 0.05.
Quality control (assay validation): The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

Example:

1. Quality control
Blank well A value: A1= 0.025 at 450nm

Well No.:	**B1**	**C1**
Negative control A values after blanking:	0.022	0.024
Well No.:	**D1**	**E1**
Positive control A values after blanking:	2.118	2.109

All control values are within the stated quality control range

2. Calculation of Nc: = $\frac{(0.022+0.024)}{2}$ = 0.023 (Nc is lower than 0.05, so take it as 0.05)

3. Calculation of the Cut-off: (C.O.) = 0.10 + 0.05 = 0.15

- The A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450 nm.
- The A values of the Positive control must be ≥ 0.800 at 450/630nm or at 450nm after blanking.
- The A values of the Negative control must be < 0.100 at 450/630nm or at 450nm after blanking.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded and the mean value calculated again using the remaining value. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Interpretations of the results
Negative Results (A/C.O. < 1)
Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no HCV has been detected with this ELISA kit. Therefore, the patient is probably not infected with HCV.

Positive Results (A/C.O. ≥ 1)
Specimens giving an absorbance equal to or higher than the Cut-off value are considered initially reactive, which indicates that Anti-HCV has probably been detected using this ELISA kit. All initially reactive specimens should be retested in duplicates using this ELISA kit before the final assay results interpretation. Repeatedly reactive specimens could be considered positive for Anti-HCV and therefore there are serological indications for infection with HCV.

Borderline (A/C.O. = 0.9-1.1)
Specimens with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results.

- If, after retesting of the initially reactive samples, both wells are negative results (A/C.O.<0.9), these samples should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step.
- If after retesting in duplicates, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for Anti-HCV.
- After retesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.

Performance characteristics
Specificity
A blood donor population of 3028 individuals was tested with 3 different kits from different manufacturers. The specificity of this anti-HCV ELISA kit was 99.56%.

Manufacturers	-	+	Confirmed positive	False Positive	Specificity
Manufacturer 1	2975	53	39	14	99.53%
Manufacturer 2	2974	54	39	15	99.50%
This assay	2976	52	39	13	99.56%

Sensitivity
Among 520 clinical hepatitis C patients confirmed positive by RIBA 3.0, 520 were positive when tested with DiaSino Anti-HCV EISA kit. **The sensitivity was 100%.**

Analytical Specificity
- No cross reactivity observed with samples from patients infected with HAV, HBV, HIV, CMV, and TP.
- No interference from rheumatoid factors up to 2000U/ml observed.
- This assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein.

Limitations
- This reagent kit is to be used for un-pooled human serum or plasma only.
- Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
- Specimens with very low level of Anti-HCV may not consistently repeat positive. In this case, it is recommended to test follow-up samples.
- Anti-HCV negative result does not preclude the possibility of infection with HCV.
- Non-repeatable false positive results may occur due to non-specific binding of the sample and conjugate to the wall of the well(s).

References

1. DiBisceglie AM. Hepatitis C and hepatocellular carcinoma. Hepatology 1997;26(3 Suppl 1):34S-38S.
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3. Maertens G, Stuyver L. Genotype and Genetic Variation of Hepatitis C Virus in The Molecular Medicine of Viral Hepatitis. Edited by Harrison TJ and Zuckerman AJ. John Wiley & Sons 1997; 183-233.
4. Nousbaum JB, Pol S, Nalpas B, et al. Hepatitis C virus type 1b (II) infections in France and Italy. Collaborative Study Group. Ann Intern Med 1995;122:161-168.
5. Gane EJ, Portmann BC, Naoumov NV, et al. Long-term outcome of hepatitis C infection after liver transplantation. N Engl J Med 1996; 334:815-820.

Symbols

IVD **In vitro diagnostic medical device**	8°C / 2°C **Temperature limit**	**Consult instructions for use**	REF **Catalog number**
LOT **Batch code**	**Date of manufacture**	**Use-by date**	Σ **Contains sufficient for <n> tests**
Manufacturer	**Do not use if package is damaged and consult instructions for use**		

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