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DIAQUICK HCV Plus

A rapid chromatographic immunoassay for the qualitative detection of antibodies to Hepatitis C virus (HCV) in human serum or plasma

REF	Content
H18200	- 25 test cassettes, individually packed in foil pouches (25x REF H18200B) with a disposable pipette and a desiccant - 1x 4 mL buffer - 1 package insert

For professional in vitro diagnostic use only.

GENERAL INFORMATION

Method	sandwich lateral flow chromatographic immunoassay
Shelf life	24 months from date of production
Storage	2-30 °C
Results	after 15 minutes at room temperature

INTENDED USE

The DIAQUICK HCV Plus is a sandwich lateral flow chromatographic immunoassay for the qualitative detection of antibodies (IgG, IgM and IgA) to Hepatitis C virus (HCV) in human serum or plasma. It is intended to be used as a screening test and as an aid in the diagnosis of infection with HCV. Any reactive specimen with the DIAQUICK HCV Plus must be confirmed with alternative testing method(s) and clinical findings.

DIAGNOSTIC SIGNIFICANCE

Hepatitis C Virus (HCV) is a small, enveloped, positive-sense, single-stranded RNA virus. Antibodies to HCV are found in over 80 % of patients with well-documented non-A, non-B hepatitis. Conventional methods fail to isolate the virus in cell culture or visualize it by electron microscope. Cloning the viral genome has made it possible to develop serologic assays that use recombinant antigens[1, 2]. Compared to the first generation HCV EIAs using single recombinant antigen, multiple antigens using recombinant protein and/or synthetic peptides have been added in new serologic tests to avoid nonspecific cross-reactivity and to increase the sensitivity of the HCV antibody tests[3, 4]. The DIAQUICK HCV Plus is a rapid test to qualitatively detect the presence of antibodies to HCV in a serum or plasma specimen. The test utilizes a combination of recombinant antigen to selectively detect elevated levels of HCV antibodies in serum or plasma.

TEST PRINCIPLE

The DIAQUICK HCV Plus is a lateral flow chromatographic immunoassay based on the principle of the double antigen-sandwich technique. The test cassette consists of: 1) a burgundy coloured conjugate pad containing HCV antigens conjugated with colloidal gold (HCV Ag conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane containing a test line region (T line) and a control line region (C line). The T line is pre-coated with non-conjugated HCV antigens, and the C line is pre-coated with goat anti-rabbit IgG. When an adequate volume of test specimen is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the membrane. HCV antibodies (IgG, IgM or IgA), if present in the specimen, will bind to the HCV Ag conjugates. The immunocomplex is then captured on the membrane by the pre-coated HCV antigens, forming a burgundy coloured T line, indicating a HCV Ab positive test result. Absence of the T line suggests a negative result. The test contains an internal control (C line) which should exhibit a burgundy coloured band of the immunocomplex of goat anti-rabbit IgG and rabbit IgG-gold conjugate regardless the presence of any antibodies to HCV. Otherwise, the test result is invalid and the specimen must be retested with another cassette.

REAGENTS

The test contains HCV antigens (including core, NS2, NS4, NS5) coated on the nitrocellulose membrane in the test line region and to colloidal gold particles on the conjugate pad. The control line region contains goat anti-rabbit IgG.
The buffer consists of casein salt: 1%, NaCl: 0.9%, Na_2HPO_4: 0.286%, NaN_3: 0.5%.

MATERIAL REQUIRED BUT NOT PROVIDED

- Clock or timer
- Specimen collection containers
- Centrifuge (for plasma only)

WARNINGS AND PRECAUTIONS

- Buffer: Warning: 0.5 % NaN_3
  H302: Harmful if swallowed
  H412: Harmful to aquatic life with long lasting effects
  P264: Wash face, hands and any exposed skin thoroughly after handling
  P280: Wear protective gloves/protective clothing/eye protection/face protection
  P260: Do not breathe dust/fume/gas/mist/vapours/spray
  P270: Do not eat, drink or smoke when using this product
  P273: Avoid release to the environment.
  P301+330+331: IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
  P314: Get medical attention/advice if you feel unwell
- For professional In Vitro diagnostic use only. Do not use after expiration date.
- Warning: the reagents in this kit contain sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides.
- When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Do not use it if the pouch is damaged or broken.
- Test is for single use only. Do not re-use under any circumstances.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout testing and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Humidity and temperature can adversely affect results.
- Do not perform the test in a room with strong air flow, ie. an electric fan or strong air conditioning.

STORAGE AND STABILITY

The kit can be stored at room temperature or refrigerated (2-30°C). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. DO NOT FREEZE. Do not use beyond the expiration date.

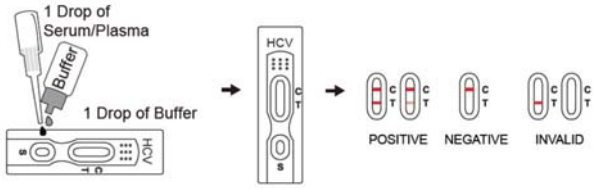
SPECIMEN COLLECTION AND STORAGE

- The DIAQUICK HCV Plus can be performed using either serum or plasma.
- For plasma: K_2EDTA, sodium heparin, sodium citrate sterile and lithium heparin should be used as the anticoagulant. Other anticoagulants have not been tested and may give incorrect results.
- Separate the serum or plasma from blood as soon as possible to avoid haemolysis. Only clear, non-haemolysed specimens can be used.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Specimens may be stored at 2-8 °C for up to 3 days. For long term storage, specimens should be kept below -20 °C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with usual regulations for transportation of etiological agents.

TEST PROCEDURE

Allow test cassette, specimen, buffer and/or controls to equilibrate to room temperature (15-30 °C) prior to testing.

1. Remove the test cassette from the foil pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
2. Place the test cassette on a clean and level surface. Hold the dropper vertically and transfer 1 drop of serum or plasma (approx. 30 µL) to the specimen well (S) of the test cassette, then add 1 drop of buffer (approx. 40 µL) and start the timer. Avoid trapping air bubbles in the specimen well (S). See the illustration below.
3. Wait for the red line(s) to appear. **The result should be read at 15 minutes.** Do not interpret the result after 30 minutes.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)

POSITIVE:* Two distinct red lines appear. One line should be in the control region (C) and another line should be in the test region (T).

***NOTE:** The intensity of the red colour in the test line region (T) will vary depending on the concentration of HCV antibodies in the specimen. Therefore, any shade of red in the test region (T) should be considered positive.

NEGATIVE: One red line appears in the control region (C). No apparent red or pink line appears in the test region (T).

INVALID: Control line fails to appear. 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 cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

A procedural control is included in the test. A red line appearing in the control region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique.

Control standards are not supplied with this kit; however it is recommended that positive and negative controls are tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

- The DIAQUICK HCV Plus is for in vitro diagnostic use only. This test should be used for the detection of antibodies to HCV in serum or plasma specimen.
- The DIAQUICK HCV Plus will only indicate the presence of antibodies to HCV in the specimen and should not be used as the sole criteria for the diagnosis of Hepatitis C viral infection.
- As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
- If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result at any time does not preclude the possibility of Hepatitis C Virus infection.
- A negative result can occur if the quantity of the antibodies to HCV present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
- Some specimens containing unusually high titre of heterophile antibodies or rheumatoid factor may affect expected results.
- As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
- Results should not be used to determine the serotype of HCV infections.
- Due to possible cross reactivity, the appearance of lines in T line does not necessarily indicate co-infection from IgG, IgM or IgA, nor can it identify the serotype.
- The recommended anticoagulants are K_2EDTA, Sodium Heparin, Sodium Citrate Sterile and Lithium Heparin. Other anticoagulants have not been evaluated with this test.

PERFORMANCE CHARACTERISTICS

Relative Sensitivity

A total of 506 HCV positive specimens were tested using the DIAQUICK HCV Plus

and a commercially available test (table 1). The relative sensitivity of the test is >99 % (95 % confidence interval: 99.27-100 %).

Table 1: Sensitivity of the DIAQUICK HCV Plus

Population	Specimen Type	No. of Tested Specimens	Positive with DIAQUICK	Positive with Competitor
Anti-HCV (any genotype)	Plasma	329	100 %	100 %
Anti-HCV (any genotype)	Serum	26	100 %	100 %
Anti-HCV (genotype 1/2/3/4 (non-subtype A)/4/5/6)	Serum / Plasma	151	100 %	100 %
Total		506	100 %	100 %

30 seroconversion panels were done:

No.	Panel	Specimen No.	Positive from days since first bleed
1	PHV907	7	0
2	PHV908	13	3
3	PHV206(M)	25	/
4	PHV911(M)	5	3
5	PHV919	7	28
6	PHV920	10 (no #2*)	16
7	HCV9047	10	28
8	HCV9046	5	69
9	HCV6229	8	17
10	HCV10041	3	6
11	HCV9041	8	62
12	HCV9045	8	37
13	HCV6222	3	40
14	HCV6224	8	19
15	HCV6227	7	75
16	HCV6228	12	31
17	HCV10071	7	84
18	HCV6220	6	18
19	HCV10185	5	130
20	HCV10235	5	96
21	HCV6215	4	20
22	HCV9042	6	8
23	HCV9058	5	10
24	HCV9094	5	9
25	HCV9095	5	10
26	HCV9055	11	65
27	HCV9054	10	72
28	HCV9044	6	21
29	HCV10165	9	19
30	HCV6226	12	39

* samples out of stock from the vendor

Relative Specificity

A total of 1259 HCV negative specimens were tested using the DIAQUICK HCV Plus and a commercially available test (Table 3). The relative specificity of the test is ＞99% (95% confidence interval: 99.71% - 100%).

Table 3: Specificity of the DIAQUICK HCV Plus

Population	Specimen Type	No. of Tested Specimens	Negative with DIAQUICK	Negative with Competitor
Clinical Negative	Serum/Plasma	202	100 %	100 %
Potentially Interfering	Serum/Plasma	30	100 %	100 %
Unselected Donors	Serum	1000	100 %	100 %
Inhibition Panel	Serum	27	100 %	100 %
Total		1259	100 %	100 %

Precision

Intra Assay: Within-run precision has been determined by using 20 replicates of four specimens: a negative, a low positive, medium positive and a high positive. The negative, low positive, medium positive and high positive values were correctly identified >99 % of the time

Inter Assay: Between-run precision has been determined by 5 independent assays on the same four specimens: a negative, a low positive, medium positive and a high positive. Three different lots of the DIAQUICK HCV Plus have been tested using negative, low positive, medium positive and high positive specimens. The specimens were correctly identified >99 % of the time.

Cross-Reactivity

No cross-reactivity was observed when samples positive for other diseases such as HIV, Syphilis, Infectious Mononucleosis, HBV, Rheumatoid Factor, HAMA, Hyper IgG, Hyper IgM, anti-HAV, anti-HSV2,anti-HEV,anti- EBV and anti-CMV were tested.

Interfering Substances

No interference was observed in samples with high concentrations of Uric acid, Ascorbic Acid, Haemoglobin, Gentisic Acid, Acetaminophen, Oxalic Acid, Albumin, Caffeine, Bilirubin, EDTA, Aspirin and Methanol.

Analytes	Conc.	Analytes	Conc.
Control	0	Control	0
Uric acid	0.15 mg/mL	Albumin	20 mg/mL
Ascorbic acid	0.2 mg/mL	Caffeine	0.2 mg/mL
Haemoglobin	5.0 mg/mL	Bilirubin	0.3 mg/mL
Gentisic acid	0.2 mg/mL	EDTA	0.2 mg/mL
Acetaminophen	1.0 mg/mL	Aspirin	0.2 mg/mL
Oxalic acid	0.2 mg/mL	Methanol	1.0 %

LITERATURE

1. Choo, Q.L., G. Kuo, A.J. Weiner, L.R. Overby, D.W. Bradley, and M. Houghton. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 189; 244: 359
2. Kuo, G., Q.L. Choo, H.J. Alter, and M. Houghton. An assay for circulating antibodies to a major etiolog Virus of human non-A, non-B hepatitis. Science 1989; 244: 362
3. Van der Poel, C.L., H.T.M. Cuypers, H.W. Reesink, and P.N. Lelie. Confirmation of hepatitis C Virus infection by new four- antigen recombinant immunoblot assay. Lancet 1991; 337: 317
4. Wilber, J.C. Development and use of laboratory tests for hepatitis C infection: a review. J. Clin. Immunoassy 1993; 16: 204

USED SYMBOLS

Symbol	Description
	Content
	Dispose of the tests and packaging appropriately

CE 0123 IVD