HEPATITIS E VIRCLIA® IgM MONOTEST

For *in vitro* diagnostic use

VCM067: Indirect chemiluminescent immunoassay (CLIA) to test IgM antibodies against hepatitis E virus in human serum/plasma. 24 tests.

INTRODUCTION:

Hepatitis E virus (HEV) is the causative agent of hepatitis E. HEV belongs to the family Hepeviridae. Four out of seven genotypes of Orthohepevirus A are known to infect humans. Genotypes 1 and 2 are responsible for human infections exclusively and are endemic in Asia, Africa and some American countries. Genotypes 3 and 4 are zoonotic and present in Europe, United States, Japan, China and Taiwan. HEV is transmitted via the fecal-oral route. Foodborne infection can occur from consumption of uncooked/undercooked meat or organs from infected animals. A wide range of clinical manifestations, from asymptomatic or subclinical to acute liver failure, can be observed, with the ratio of symptomatic to asymptomatic infection ranging from 1:2 to 1:13. When they occur, the signs and symptoms of hepatitis E develop between 15 and 60 days after exposure and are similar to those of other types of acute viral hepatitis, including jaundice, anorexia, abdominal pain, and hepatomegaly. HEV is also responsible for extrahepatic disorders: neurological syndromes, renal injury, pancreatitis, and hematological disorders. Although infection is usually mild without sequelae, it can be a serious illness for pregnant women, persons with preexisting chronic liver disease and immunocompromised patients. Differential diagnosis is achieved by testing serum samples with specific assays for viral genome amplification and by methods for detecting anti-HEV IgM. Additionally, anti-HEV IgG can be used to assess the prevalence of HEV infection among a population. Antibodies to HEV are usually positive after 2-3 weeks after exposure. Anti-HEV IgM declines rapidly after early convalescence and is usually negative by 13 weeks, falling below the cutoff level among most patients after 32 weeks. IgM response is weak and delayed, even undetectable, in immunocompromised patients. Anti-HEV IgG develops early after clinical onset of hepatitis E and is long lasting.

Detection methods based on chemiluminescence have received much attention due to their low background, linearity and wide dynamic range. When coupled to enzyme immunoassays, the signal amplification effect provided by the enzyme enables the design of CLIA (**C**hemi**L**uminescent **I**mmuno**A**ssay) tests with shorter incubation times while keeping or improving their sensitivity.

PRINCIPLE OF THE TEST:

The CLIA method is based upon the capture of IgM in the sample with anti-IgM antibodies adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. Then the antigen labeled with peroxidase reacts with the IgM captured, and the unbound is removed by washing; bound conjugate is developed with the aid of a chemiluminescent substrate solution that will generate a glow-type luminescence that can be read with a luminometer.

KIT FEATURES:

All reagents supplied are ready to use.

Serum dilution solution and conjugate are coloured to help in the performance of the technique.

Sample predilution is not necessary.

Reagents required for the run of the test are included in the monodose presentation.

KIT CONTENTS:

1 VIRCLIA® HEPATITIS E IgM MONODOSE: 24 monodoses consisting of 3 reaction wells and 5 reagent wells with de following composition:

Wells A, B, C: reaction wells; wells coated with anti-IgM antibodies (µ-specific).

Well D: Conjugate: orange; containing HEV recombinant antigen, peroxidase conjugate dilution and Neolone and Bronidox as preservatives.

Well E: Serum dilution solution: blue; phosphate buffer containing protein stabilizers and Neolone and Bronidox as preservatives.

Well F: Calibrator: clear; positive serum dilution containing Neolone and Bronidox as preservatives.

Well G: Substrate component B: clear; containing peroxide.

Well H: Substrate component A: clear; containing luminol.

Store at 2-8ºC and check expiration date.

Materials required but not supplied:

-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR)

-Precision micropipettes 5 and 100 µl.

-Eight channel micropipette 100 µl.

-Adapted microplate washer.

-Thermostatized incubator/water bath.

-Microplate luminometer.

-Alternatively, a CLIA automated processor.

STORAGE REQUIREMENTS:

Store at 2-8ºC. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored, closed and at 2-8ºC.

STORAGE OF REAGENTS ONCE OPENED:

Reagent	Stability
VIRCLIA® MONODOSE	Once opened, use it in the same day

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.

Do not let the plate dry between washing and reagent addition.

Substrate component A is light sensitive. Avoid light exposure. Substrate solution should not get in contact with acid, combustible materials and strong oxidizing or reducing agents. Make sure that no metal components come in contact with the substrate without having previously tested their compatibility.

VIRCELL, S.L does not accept responsibility for the mishandling of the reagents included in the kit.

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RECOMMENDATIONS AND PRECAUTIONS:

1. For *in vitro* diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only components of the AUXILIARY REAGENTS kit are compatible with all VIRCLIA® references and lots.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens. Wash hands thoroughly after manipulating samples. Besides, follow all safety protocols in use in your laboratory.
5. Do not use in the event of damage to the package.
6. Never pipette by mouth.
7. Serum dilution solution, reaction wells, conjugates and calibrator in this kit include substances of animal origin. Calibrator includes as well substances of human origin. Although the human serum controls of this kit have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. Reaction wells are coated with inactivated antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
8. Substrate solution may be irritant to eyes, respiratory system and skin. In case of contact with this solution, rinse thoroughly with water and seek medical attention. For further information a Material Safety Data Sheet is available.
9. Do not use this product in automated processors unless they have been previously validated for that purpose.

SPECIMEN COLLECTION AND HANDLING:

Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum/plasma samples are to be refrigerated (2-8ºC) upon collection or frozen (-20ºC) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated samples. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

PRELIMINARY PREPARATION OF THE REAGENTS:

All reagents supplied are ready to use.
Only the VIRCLIA® WASHING SOLUTION included in the auxiliary component kit VIRCLIA® AUXILIARY REAGENTS must be prepared in advance. Fill 50 ml of VIRCLIA® WASHING SOLUTION (20x) up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37ºC before diluting. Once diluted, store at 2-8ºC.

ASSAY PROCEDURE:

- **AUTOMATED**

1. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
2. Follow the Operator´s Manual of the Automated Processor.

- **MANUAL**

Contact the manufacturer for further information on the manual procedure.

INTERNAL QUALITY CONTROL:

Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.
The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS:

Each monodose includes one calibrator (well A) and one dilution of the calibrator used as negative control (well C). It allows the validation of the assay and kit.

RLU of the calibrator and the negative control must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

Control	RLU
CALIBRATOR	2-7
NEGATIVE CONTROL	<2

INTERPRETATION OF RESULTS:

Antibody index= (sample RLU/calibrator RLU)

Index	Interpretation
<0.4	Negative
0.4-0.5	Equivocal
>0.5	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.
Samples with indexes below 0.4 are considered as not having antibodies of the specificity and class measured by this kit.
Samples with indexes above 0.5 are considered as having antibodies of the specificity and class measured by this kit.

LIMITATIONS:

1. This kit is intended to be used with human serum/plasma.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by isolation techniques.
4. This test will not indicate the site of infection. It is not intended to replace isolation.
5. Lack of significant rise in antibody level does not exclude the possibility of infection.
6. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended an IgM assay be performed or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.
7. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.

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8. The results of a single-specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.
9. The performance results showed correspond to comparative studies with commercial predicative devices in a defined population sample. Small differences can be found with different populations or different predicative devices.

PERFORMANCES:

- **SENSITIVITY AND SPECIFICITY:**

256 serum/plasma samples were assayed against a commercial ELISA kit. The results were as follows:

Samples No.	Sensitivity	Specificity
256	95%	99%

Indeterminate values were omitted from the final calculations.

- **INTRA-ASSAY PRECISION:**

3 sera were individually run 10 times each serum in a single automated assay in essentially unchanged conditions.
The results were as follows:

Serum	N	% C.V.
Sample +	10	5
CAL	10	7
CN	10	6

C.V. Coefficient of variation

- **INTER-ASSAY PRECISION:**

3 sera were individually run on 5 consecutive days in 2 different automatic processors.
The results were as follows:

Serum	N	% C.V.
Sample +	10	6
CAL	10	16
CN	10	15

C.V. Coefficient of variation

- **CROSS REACTIVITY AND INTERFERENCES:**

51 samples known to be positive for other microorganisms (hepatitis A, hepatitis B, hepatitis C, Epstein-Barr virus VCA, cytomegalovirus) were assayed. 20 samples known to be positive for rheumatoid factor and antinuclear antibodies were assayed.
No cross-reactivity with hepatitis A (10 tested samples), hepatitis B (10 tested samples), hepatitis C (10 tested samples) and Epstein-Barr VCA (10 tested samples) was found. Cross reactivity with cytomegalovirus (1 out of 11 tested samples) was found. No interferences with rheumatoid factor (10 samples tested) were found. No interferences with antinuclear antibodies specimens (10 samples tested) were found.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
	Store at x-yºC
Σ n	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS X	<X> wells

BIBLIOGRAPHY:

1. Kamar N, Dalton HR, Abravanel F, Izopet J. 2014. Hepatitis E virus infection. Clin Microbiol Rev. 27(1):116-38.
2. Avellon A, Morago L, Garcia-Galera del Carmen M, Munoz M, Echevarría JM. 2015. Comparative sensitivity of commercial tests for hepatitis E genotype 3 virus antibody detection. J Med Virol. 87(11):1934-9.
3. Echevarría JM, González JE, Lewis-Ximenez LL, Dos Santos DR, Munné MS, Pinto MA, Pujol FH, Rodríguez-Lay LA. 2013. Hepatitis E virus infection in Latin America: a review. J Med Virol.;85(6):1037-45.
4. Aspinall EJ, Couturier E, Faber M, Said B, Ijaz S, Tavoschi L, Takkinen J, Adlhoch C; country experts. 2017. Hepatitis E virus infection in Europe: surveillance and descriptive epidemiology of confirmed cases, 2005 to 2015. Euro Surveill. 29;22(26). pii: 30561.
5. Norder H, Karlsson M, Mellgren Å, Konar J, Sandberg E, Lasson A, Castedal M, Magnius L, Lagging M. 2016. Diagnostic Performance of Five Assays for Anti-Hepatitis E Virus IgG and IgM in a Large Cohort Study. J Clin Microbiol. 54(3):549-55.
6. Clemente-Casares P, Ramos-Romero C, Ramirez-Gonzalez E, Mas A. 2016. Hepatitis E Virus in Industrialized Countries: The Silent Threat. Biomed Res Int. 2016:9838041.
7. CDC. Viral hepatitis. Available from http://www.cdc.gov/. [8 August 2017].
8. Velan, B., M. Halmann. 1978. Chemiluminescence immunoassay. A new sensitive method for determination of antigens. Immunochemistry. 15: 331–333.
9. Whitehead, T.P., L.J. Kricka, T.J. Carter, G.H. Thorpe. 1979. Analytical luminescence: its potential in the clinical laboratory. Clin Chem. 25:1531-46.
10. Zhao, L., L. Sun, X. Chu. 2009. Chemiluminescence immunoassay. TrAC-Trend Anal Chem. 28: 404–415.

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