# HEV ELISA 4.0

## Instructions for Use

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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MBE0012-ENG-0

23542-096: (96 tests)

### NAME AND INTENDED USE

The **MP DIAGNOSTICS (MPD) HEV ELISA 4.0** is an enzyme-linked immunosorbent assay intended for the detection of total antibodies to Hepatitis E Virus in human serum or plasma.

This kit is supplied for Research Use Only. It is not intended for use in the diagnosis or prognosis of disease.

### INTRODUCTION

Hepatitis E Virus (HEV) is a single-stranded, positive sense, non-enveloped RNA virus, which was first identified as an enterically-transmitted non-A, non-B hepatitis virus by Genelabs in 1990 (1,2). The course of the infection of HEV is generally acute and self-limiting without chronic sequelae. There is, however, a high incidence of mortality in pregnant women in the third trimester, about 10-20% (3) and a mortality rate of 1-2% in the general population, which is 10 times that of hepatitis A (HAV). With the cloning of the etiological agent of ET-NANBH at Genelabs and the identification of type common viral epitopes (1,2), specific diagnostic tools have been developed to detect antibodies to HEV.

Major epidemics of enterically transmitted non-A, non-B hepatitis (ET-NANBH) have been found to occur in developing regions such as Asia, the former USSR, Central America and Africa (3,4). Sporadic cases have been reported in developed nations, including Australia, the United Kingdom and the United States (5,6,7). Cases in developed nations have generally been associated with travel to endemic regions. However, accumulated evidences suggest that sporadic cases of HEV infections without an association with endemic regions also occur in a wide range of non-endemic areas, including Western Europe, Greece, United States, Australia, and Taiwan (8-17).

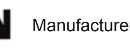
It has been demonstrated in the experiments that human HEV is capable of infecting animal species (18-21), while non-human primate may get infected with swine HEV (21). Recent studies on prevalence of HEV infection in animal show the high seroprevalence of antibody to HEV in different animal species, including swine, equine, roden, etc. Mounting evidences indicate that wide spread of HEV infection in animals, in particular swines, could represent an important reservoir for virus transmission. Some of the sporadic cases of HEV infection in non-endemic areas may be attributed to zoonotic transmission.

A HEV ELISA that is highly sensitive and specific is needed for the detection of HEV total antibodies in human serum or plasma.

The **MPD HEV ELISA 4.0** utilises a proprietary recombinant antigen, which is highly conserved between different HEV strains (22,23,24), to detect the presence of specific antibodies including IgG, IgM and IgA against HEV.

### DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on MP Diagnostics products and packaging. These symbols are the most common ones appearing on medical devices and their packaging. They are explained in more detail in the European Standard EN 980:2008 and International Standard ISO 15223-1:2007.

| Symbol | Meaning | Symbol | Meaning |
|---|---|---|---|
| | Use by | | Consult Instructions for Use |
| | Batch Code | | Catalogue Number |
| | Temperature Limitation | | Caution |
|  | Manufacturer | | Contents |
| | Sufficient for <n> tests | | Harmful (Xn) / Irritant (Xi) |
| | Do not reuse | | Corrosive |

### CHEMICAL & BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The wells of the polystyrene microplate strips are coated with a proprietary recombinant antigen presenting a conformational epitope that is highly conserved between different HEV strains. The HRP conjugate is produced with the same recombinant antigen labeled with horseradish peroxidase. This conjugate is first diluted appropriately in diluent buffer prior to being dispensed into the antigen-coated wells of the microplates. Serum or plasma samples are then added to the antigen-coated wells containing the diluent buffer and the conjugate. After incubation, HEV specific antibodies (IgG, IgM and IgA), if present, will bind to both the antigens immobilised on the wells and the antigen of the conjugate in the diluent. Subsequently, the wells are thoroughly washed to remove the unbound materials. A substrate solution containing 3,3',5,5'-tetramethylbenzidine (TMB) is then added to each well. The presence of specific antibodies is indicated by the presence of blue colour solution after incubation. Reaction is terminated by addition of sulphuric acid. The colour intensity of the resulting yellow reaction product is measured at 450nm using microplate reader and its corresponding optical density or absorbance is proportional to the amount of antibodies present in the specimen.



### KIT COMPONENTS

| | Component Description | Quantity Provided |
|---|---|---|
| MICROPLATE | **HEV MICROPLATE** Twelve 8-well strips per plate, sealed in an aluminum pouch with desiccant. Each microplate well contains adsorbed recombinant HEV protein. Store at 2°C to 8°C. | 1 plate (96 tests) |
| CONTROL − | **NON-REACTIVE CONTROL** Inactivated normal human serum, non-reactive for anti-HCV, anti-HEV, HBsAg and anti-HIV-1. Contains thimerosal and sodium azide as preservatives. Store at 2°C to 8°C. | 1 vial (400µl) |
| CONTROL + | **REACTIVE CONTROL** Inactivated human serum containing a high titer of IgG antibodies specific for HEV. Contains thimerosal and sodium azide as preservatives. Store at 2°C to 8°C. | 1 vial (400µl) |
| DILUENT | **SAM DILUENT (SAM = Sample Addition Monitor)** Tris based saline solution containing heat-treated normal goat serum, bovine serum albumin and stabilizers. Contains BRONIDOX®L as preservative. Store at 2°C to 8°C. | 1 bottle (100ml) |
| WASH PLATE 20x | **PLATE WASH CONCENTRATE (20x)** Phosphate buffered saline with Tween-20. Contains chloroacetamide as preservative. Store at 2°C to 8°C. | 1 bottle (120ml) |
| CONJUGATE | **CONJUGATE** HEV antigen labeled with horseradish peroxidase. Contains 0.02% thimerosal as preservative. Store at 2°C to 8°C. | 1 vial (50µl) |
| SUBS TMB | **SUBSTRATE BUFFER** Buffer containing 3,3',5,5'-tetramethylbenzidine (TMB). Store in the dark at 2°C to 8°C. | 1 bottle (12.5ml) |
| SOLN STOP $H_2SO_4$ 2M | **STOP SOLUTION** 2M sulphuric acid solution. Store at 2°C to 8°C. | 1 bottle (30ml) |
| | **PLATE COVERS** Adhesive covers for microplate during incubation. | 4 pieces |
| | **INSTRUCTIONS FOR USE** | 1 copy |

### WARNINGS AND PRECAUTIONS

1. For Research Use only. It is not intended for use in diagnostic procedure.
2. Please refer to the product labeling for information on potentially hazardous components.

**HEALTH AND SAFETY INFORMATION**

**CAUTION**: This kit contains materials of human origin. No test method can offer complete assurance that human blood products will not transmit infection.

**HANDLE ASSAY SPECIMENS, REACTIVE AND NON-REACTIVE CONTROLS AS POTENTIALLY INFECTIOUS AGENTS.** It is recommended that the components and test specimens be handled using good laboratory working practices. They should be disposed of in accordance with established safety procedures.

The ***Reactive Control*** and ***Non-Reactive Control*** contain 0.005% Thimerosal and 0.1% Sodium Azide. Sodium Azide can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small, nevertheless when disposing of azide-containing materials they should be flushed away with relatively large quantities of water to prevent metal azide buildup in plumbing system. The following are the appropriate Risk (R) and Safety (S) phrases.

**Thimerosal:**
**R26/27/28** Very toxic by inhalation.
**R27** Very toxic by contact with skin.
**R28** Very toxic if swallowed.
**S28** After contact with skin, wash immediately with plenty of water.
**S36** Wear suitable protective clothing.
**S45** In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

**Sodium Azide:**
**R28** Very toxic if swallowed.
**R32** Contact with acids liberates very toxic gas.
**S28** After contact with skin, wash immediately with plenty of water.
**S45** In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

The ***Diluent*** contains 0.5% BRONIDOX®L, which is classified per applicable European Economic Community (EEC) Directives as Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

**R22** Harmful if swallowed.
**R38** Irritating to skin.
**S36** Wear suitable protective clothing.
**S46** If swallowed, seek medical advice immediately and show this container or label.

The ***Plate Wash Concentrate (20x)*** contains 2% Chloroacetamide which is classified per applicable European Economic Community (EEC) Directives as Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

**R25** Toxic if swallowed.
**R43** May cause sensitization by skin contact.
**R62** Possible risk of impaired fertility.
**S22** Do not breathe dust.
**S36** Wear suitable protective clothing.
**S37** Wear suitable gloves.
**S45** In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).



The **Stop Solution** is 2M Sulphuric Acid which is classified per applicable European Economic Community (EEC) Directives as corrosive (C). The following are the appropriate Risk (R) and Safety (S) phrases.

**R35** Causes severe burns.
**S26** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
**S30** Never add water to this product.
**S45** In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

1. Avoid microbial contamination of reagents when opening and removing aliquots from the original vials or bottles.
2. Do not pipette by mouth.
3. Handle assay specimens, microplates, Reactive and Non-Reactive Controls as potentially infectious agents.
4. Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in bio-hazard waste-bags. Wash hands thoroughly afterwards.
5. It is highly recommended that this assay be performed in a biohazard cabinet.
6. Keep materials away from food and drink.
7. In case of an accident or contact with eyes, rinse immediately with plenty of water and seek medical advice.
8. Consult a physician immediately in the event that contaminated materials are ingested or come in contact with open lacerations, or other breaks in the skin.
9. Sulphuric acid can cause burns. **AVOID CONTACT**. If it comes into contact with skin, wash thoroughly with water.
10. Avoid contact of sulphuric acid with any oxidizing agent or metal.
11. Do not expose substrate to strong light.
12. Wipe spills of potentially infectious materials immediately with absorbent paper and swab the contaminated area with an effective disinfecting agent before work is resumed.

### ANALYTICAL PRECAUTIONS

1. Use only sera or plasma samples collected in EDTA, Heparin, Sodium Citrate, K-Oxalate or Acid Citrate Dextrose (ACD). Before storage, ensure that blood clot or blood cells have been separated by centrifugation.
2. Do not use whole blood or other body fluids.
3. Optimal assay performance requires **STRICT ADHERENCE** to the assay procedure described in this Instructions for Use. Deviations from the procedure may lead to aberrant results.
4. **DO NOT MODIFY OR SUBSTITUTE REAGENTS FROM ONE KIT LOT TO ANOTHER.** Controls, conjugate and microplates are matched for optimal performance. Use only the reagents supplied with the kit.
5. Do not use kit components beyond the expiry date printed on the kit box.
6. Avoid microbial contamination of the reagents, when opening and removing aliquots from the original vials or bottles. As this will prematurely reduce the shelf life of the kits and give erroneous results. Use aseptic techniques including pipettes or disposable pipette tips when drawing aliquots from vials.
7. To prevent cross contamination, use a new pipette tip for each specimen aliquoted to, and do not touch the top or the bottom of the strips, the edge of the wells or the liquid in the wells with fingers or pipette tips.
8. It is recommended that glassware to be used with the reagents should be washed with 2M hydrochloric acid and rinsed thoroughly with distilled or deionised water prior to use.
9. For best results, equilibrate all reagents and test specimens to room temperature (25°C ± 5°C) before use. Immediately after use, return to 2°C to 8°C storage.
10. Use only reagent grade quality, deionised or distilled water to dilute reagents.
11. **ALL REAGENTS MUST BE MIXED WELL BEFORE USE.**
12. **WORKING CONJUGATE SOLUTION SHOULD BE PREPARED FRESH PRIOR TO USE.**
13. Do not expose reagents or perform test in an area containing a high level of chemical disinfectant fumes (e.g. hypochlorite fumes) during storage or during incubation steps. Contact inhibits colour reaction. Also do not expose reagents to strong light.
14. Do not remove microplates from the storage bag until immediately before use. Opened, unused strips should be stored at 2°C to 8°C in its storage bag with the desiccant provided.
15. The kit controls should be assayed concurrently with test specimens for each test run.
16. Care should be taken to avoid touching or splashing the rim of the well with conjugate. Do not "blow out" from the micropipette. It is recommended to use reverse pipetting whenever possible.
17. Use of highly haemolyzed samples, incomplete clotted sera, plasma samples containing fibrin or samples with microbial contamination may cause erroneous results.
18. Do not use a water bath to incubate microplates.
19. During 37°C incubation, evaporation must be prevented. Cover plates with adhesive covers provided.
20. Avoid repeatedly opening and closing the incubator door during incubation steps.
21. Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Remove any bubbles in the well, e.g. by gentle tapping.
22. Ensure that automated equipment is validated before use.
23. Routine maintenance of aspiration / wash system is strongly recommended to prevent carryover from highly reactive specimens to non-reactive specimens.

### STORAGE

1. Store **MPD HEV ELISA 4.0** kit and its components at 2°C to 8°C when not in use.
2. All test reagents and strips in the closed or unopened condition, when stored at 2°C to 8°C, are stable until the expiry date given on the kit. Do not freeze the reagents.
3. Crystals may form when Plate Wash Concentrate (20x) is stored at 2°C to 8°C. These must be dissolved by warming at 37°C prior to use.
4. Precipitate may form when the Diluent is stored at 2°C to 8°C. This will not affect the performance of the kit.

### SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Serum or plasma specimens collected in EDTA, heparin, sodium citrate, K-oxalate or ACD may be used. Before storage, ensure that blood clot or blood cells have been separated by centrifugation.

Fresh specimens are preferred, specimens that undergo freeze-thaw cycles repeatedly are not recommended. Specimens should be stored at 2°C to 8°C if the test is to be run within 7 days of collection or frozen at ≤ -20°C if the test is to be delayed for more than 7 days. In addition, up to 0.1% Sodium Azide may be used to stabilize serum or plasma specimens stored at 2°C to 8°C.

Clear, non-haemolysed samples are preferred. Lipemic, icteric or contaminated (particulate) samples should be filtered (0.45µm) or centrifuged before testing.

Samples can be inactivated but this is not a requirement for optimal test performance.

Inactivate as follows:
1. Loosen cap of sample container.
2. Heat-inactivate sample at 56°C for 30 minutes in a water bath.
3. Allow sample to cool down before retightening cap.
4. Sample can be stored frozen until analysis.

Repeated freeze-thawing of sample is not recommended.

### ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. Disposable absorbent bench top paper and paper towels.
2. Polypropylene tubes or containers.
3. Graduated pipettes: 5ml, 10ml.
4. Multichannel pipettor capable of delivering 20µl,100µl, and 200µl.
5. Pipettor capable of delivering 1-1000µl.
6. Disposable pipette tips.
7. Reagent reservoirs (troughs) with a capacity of 25ml.
8. Deionised or distilled water, reagent grade quality.
9. Flasks: 500ml, 1 litre.
10. ELISA Microplate Washer. Alternatively, washing can be performed manually by using a multichannel pipettor delivering 0.3ml volumes and an aspirator device.
11. A 37 ± 1°C incubator.
12. A dual ($A_{450}$–$A_{620}$) or single ($A_{450}$) wavelength microplate reader.
13. Effective disinfecting agent.

### PREPARATION OF REAGENTS

1. **WORKING CONJUGATE**
   a. **WORKING CONJUGATE** should be **prepared fresh prior to use**.
   b. Mix **CONJUGATE** and **DILUENT** thoroughly before use. **DO NOT SPIN** the mixture.
   c. Dilute **CONJUGATE** at 1:500 dilution factor with **DILUENT.** For example, add 6.0µl conjugate into 3.0ml diluent.
   d. Use only polypropylene containers or tubes.
   e. 9.0ml of **WORKING CONJUGATE** is required for one microplate.



| CONJUGATE PREPARATION CHART (1:500 dilution factor) | | |
|---|---|---|
| Number of tests | Vol. of Conjugate (µl) | Vol. of Diluent (ml) |
| 24 | 6.0 | 3.0 |
| 48 | 10.0 | 5.0 |
| 72 | 14.0 | 7.0 |
| 96 | 18.0 | 9.0 |

2. **DILUTED WASH BUFFER**
   a. DILUTED WASH BUFFER should be **prepared fresh prior to use.**
   b. Dilute 1 volume of PLATE WASH CONCENTRATE with 19 volumes of distilled water (reagent grade quality). Mix well. Approximately 200ml of wash buffer is required to wash 1 plate.

### ASSAY PROCEDURE

**IMPORTANT**: - Immunoassays of this nature are temperature-sensitive and time-dependent. Strict adherence to the assay procedure will ensure optimal assay performance. Deviations from the recommended procedure may lead to aberrant results.

1. Prepare **WORKING CONJUGATE** as described in the **PREPARATION OF REAGENTS**.
2. Remove microplate from the aluminum bag.
3. Shake specimen and control vials before use.
4. Fill a reagent reservoir with **WORKING CONJUGATE**. Using a multichannel pipettor, add 80µl of **WORKING CONJUGATE** to all wells. — 80µl
5. Wells A1 and B1 are '**BLANKS**'. **DO NOT ADD SPECIMEN TO THESE WELLS.** Add 20µl of diluent per well to these wells. — 20µl
6. Add 20µl of specimen to the assigned well, starting at well A2. This will give a final specimen dilution of 1:5. Mix by pipetting up and down once. **DO NOT PLACE SPECIMEN IN AN EMPTY WELL.** — 20µl
7. After the test specimen have been added, add 20µl of **NON-REACTIVE CONTROL** per well to wells C1, D1 and E1. — 20µl
8. Add 20µl of **REACTIVE CONTROL** per well to wells F1, G1 and H1. Mix thoroughly by tapping gently on all sides of microplate, taking care to keep the plate flat on the bench-top. — 20µl

9. Carefully cover the microplate with a plate cover provided to prevent evaporation during incubation.

10. **Incubate for 60 minutes at 37°C (Do not use a 37°C water bath for incubation).** — 60 min

11. Remove and discard the plate cover and wash the microplate with **DILUTED WASH BUFFER** using one of the two recommended methods. — 300µl per well per wash

    A. Automated or Semi-automatic Microplate Washer - Wash six (6) times with at least 300µl per well per wash.

    B. Manual Microplate Washer – Aspirate completely the contents of all wells by lowering the aspirator tip gently to the bottom of each well. **BE CAREFUL NOT TO SCRATCH THE INSIDE OF THE WELL SURFACE.** Fill the entire plate with at least 300µl per well, then aspirate immediately in the same order. Perform this cycle six (6) times.

12. Blot dry by inverting the microplate and tapping firmly onto absorbent paper. All residual plate wash buffer should be blotted dry. Colour formation can be inhibited during the substrate incubation by residual plate wash buffer.

13. Fill a reagent reservoir with **SUBSTRATE**. Using a multichannel pipettor, add 100µl of **SUBSTRATE** to each well. Apply a plate cover. — 100µl

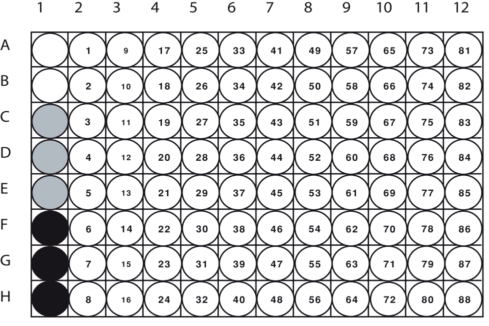
14. Incubate for 30 minutes in the dark at 37°C. **(Do Not use a 37°C water bath for incubation).** — 30 min

15. Remove and discard the plate cover.

16. Using a multichannel pipettor, add 50µl of **STOP SOLUTION** to each well. Mix gently by tapping the plate. — 50µl

17. Determine the absorbance for each well at 450nm. If a dual filter instrument is used, the reference wavelength should be 620nm.

**NOTE: Absorbance should be read within 10 minutes upon addition of the STOP SOLUTION.**




## QUALITY CONTROL

1. The BLANK should be assayed in duplicate, whereas NON-REACTIVE CONTROL and REACTIVE CONTROL in triplicate on each plate with each run of specimens.
2. Blank values must have an absorbance of ≤ 0.100.
3. Non-Reactive Control values must have an absorbance of ≤ 0.100.
4. At least 2 of the 3 Reactive Control values must have absorbance ≥ 0.500. Any values outside of this range should not be used for calculation of the Reactive Control Mean (RCx̄).

## RESULTS

Each microplate must be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed.

The presence or absence of antibodies specific for HEV is determined by relating the absorbance of the specimens to the CUT-OFF VALUE (COV) of the plate.

**The CUT-OFF VALUE is calculated as (0.40 absorbance unit + NRC Mean Absorbance):**

CUT-OFF VALUE = 0.40 + NRCx̄



## CALCULATION OF RESULTS

**1. Calculation of Non-Reactive Control Mean Absorbance (NRCx̄)**

Example:

| Well No. | Absorbance |
|---|---|
| C1 | 0.050 |
| D1 | 0.051 |
| E1 | 0.052 |
| Total | 0.153 |
| Mean | 0.153 / 3 = 0.051 (NRCx̄) |

Individual Non-Reactive Control values should be ≤ 0.100 unit.

If one Non-Reactive Control value does not meet the above criteria, it must be excluded as aberrant. The Non-Reactive Control Mean (NRCx̄) should then be recalculated using the remaining individual Non-Reactive Control values. All remaining individual Non-Reactive Control values must meet the above criteria or the assay is invalid and must be repeated.

**2. Calculation of Reactive Control Mean Absorbance (RCx̄)**

Example:

| Well No. | Absorbance |
|---|---|
| F1 | 1.221 |
| G1 | 1.144 |
| H1 | 1.298 |
| Total | 3.663 |
| Mean | 3.663 / 3 = 1.221 ( RCx̄) |

Individual Reactive Control values must be ≥ 0.500 unit.

If one Reactive Control value does not meet the above criteria, it must be excluded as aberrant. The Reactive Control Mean (RCx̄) should then be recalculated using the remaining individual Reactive Control values. All remaining individual Reactive Control values must meet the above criteria or the assay is invalid and must be repeated.

**3. Calculation of the difference between RCx̄ and NRCx̄**

Example:
NRCx̄ = 0.051
RCx̄ = 1.221
RCx̄ - NRCx̄ = 1.221 - 0.021
= 1.200

For the assay to be valid, the RCx̄ - NRCx̄ value should be ≥ 0.500. If not, improper technique or deterioration of reagents may be suspected and the assay should be repeated.

**4. Calculation of CUT - OFF value**

CUT - OFF Value = 0.40 + NRCx̄
Example: NRCx̄ = 0.051
CUT - OFF Value = 0.40 + 0.051
= 0.451

## LIMITATIONS OF THE METHOD

Deviation from the recommended procedure may lead to aberrant results.

## LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no express warranty other than that the test kit will function as a *Research Use* only assay within specifications and limitations described in the product Instructions for Use when used in accordance with the instructions contained therein. The manufacturer disclaims any warranty express or implied, including such express or implied warranty with respect to merchantability, fitness for use or implied utility for any other purposes. The manufacturer is limited to either replacement of the product or refund of the purchase price of the product. The manufacturer shall not be liable to the purchaser or third parties for any damage, injury or economic loss howsoever caused by the product in the use or in the application thereof. The manufacturer makes no representation express or implied, that this product will not infringe the intellectual property rights of the third parties.

## TECHNICAL PROBLEMS/COMPLAINTS

Should there be a technical problem / complaint, please do the following:
1. Note the kit lot number and the expiry date.
2. Retain the kits and the results that were obtained.
3. Contact the nearest MP Biomedicals office or your local distributor.



## BIBLIOGRAPHY


1. Reyes, G.R., M.A. Purdy, J.P. Kim, K.C. Luk, L.M. Young, K.E. Fry, and D. Bradley. Isolation of a cDNA from the virus responsible for enterically-transmitted non-A, non-B hepatitis. 1990. Science. 247: 1335-1359.
2. Yarbough, P.O., A.W. Tam, K.E. Fry, K. Krawczynski, K.A. McCaustland, D.W. Bradley and G.R. Reyes. Hepatitis E Virus: Identification of type-common epitopes. J Virol. 1991. 65(11): 5790-5797.
3. Bradley, D.W. 1990. Enterically-transmitted non-A, non-B hepatitis. pp 442-461. In A.J. Zuckerman (ed) British Medical Bulletin 46(2). Churchill Livingstone, New York.
4. Purcell, R.H. and J.R. Ticehurst. 1988. Enterically transmitted non-A, non-B hepatitis: Epidemiology and clinical characteristics. pp. 131-137. In A.J. Zuckerman (ed). Viral Hepatitis and Liver Disease. Alan R. Liss Inc., New York.
5. Moaven, L.D., A.J. Fuller, J.C. Doultree, J.A. Marshall, D.S. Bowden, R.A. Moeckli and S.A. Locarnini. 1993. A case of acute Hepatitis E in Victoria. Medical Journal of Australia. 159; 124-125.
6. Skidmore, S.J., P.O. Yarbough, K.A. Gabor, A.W. Tam, G.R. Reyes, A.J.E. Flower. Imported Hepatitis E in UK. The Lancet. 1991. 337; 1541.
7. Dawson, G.J., I.K. Mushahwar, K.H. Chau, G. L. Gittnick. Detection of long-lasting antibody to Hepatitis E Virus in a US traveller to Pakistan. The Lancet. 1992. 340; 426.
8. Pavia M, Iiritano E, Veratti MA, Angelillo IF. Prevalence of Hepatitis E antibodies in healthy persons in southern Italy. Infection. 1998. 26(1): 32-35.
9. Pina S, Jofre J, Emerson SU, Purcell RH, Girones R. Characterization of a strain of infectious Hepatitis E Virus isolated from sewage in an area where Hepatitis E is not endemic. Appl Environ Microbiol. 1998. 64(11): 4485-4488.
10. Sylvan SP, Jacobson SH, Christenson B. Prevalence of antibodies to Hepatitis E Virus among hemodialysis patients in Sweden. J Med Virol. 1998. 54(1): 38-43.
11. McCrudden R, O'Connell S, Farrant T, Beaton S, Iredale JP, Fine D. Sporadic acute Hepatitis E in the United Kingdom: an underdiagnosed phenomenon? Gut. 2000. 46(5): 732-733.
12. Dalekos GN, Zervou E, Elisaf M, Germanos N, Galanakis E, Bourantas K, Siamopoulos KC, Tsianos EV. Antibodies to Hepatitis E Virus among several populations in Greece: increased prevalence in an hemodialysis unit. Transfusion. 1998. 38(6): 589-595.
13. Mateos ML, Camarero C, Lasa E, Teruel JL, Mir N, Baquero F. Hepatitis E Virus: relevance in blood donors and other risk groups. Vox Sang. 1998. 75(4): 267-269.
14. Schlauder GG, Dawson GJ, Erker JC, Kwo PY, Knigge MF, Smalley DL, Rosenblatt JE, Desai SM, Mushahwar IK. The sequence and phylogenetic analysis of a novel Hepatitis E Virus isolated from a patient with acute hepatitis reported in the United States. J Gen Virol. 1998. 79(Pt 3): 447-456. Erratum in: J Gen Virol. 1998. 79(Pt 10): 2563.
15. Tsang TH, Denison EK, Williams HV, Venczel LV, Ginsberg MM, Vugia DJ. Acute Hepatitis E infection acquired in California. Clin Infect Dis. 2000. 30(3): 618-619.
16. Heath TC, Burrow JN, Currie BJ, Bowden FJ, Fisher DA, Demediuk BH, Locarnini SA, Anderson DA. Locally acquired Hepatitis E in the Northern Territory of Australia. Med J Aust. 1995. 162(6): 318-319.
17. Wu JC, Chen CM, Chiang TY, Sheen IJ, Chen JY, Tsai WH, Huang YH, Lee SD. Clinical and epidemiological implications of swine Hepatitis E virus infection. J Med Virol. 2000. 60(2): 166-171.
18. Balayan MS, Usmanov RK, Zamyatina NA, Djumalieva DI, Karas FR. Brief report: experimental Hepatitis E infection in domestic pigs. J Med Virol. 1990. 32(1): 58-59.
19. Usmanov RK, Balaian MS, Dvoinikova OV, Alymbaeva DB, Zamiatina NA, Kazachkov IuA, Belov VI. An experimental infection in lambs by the Hepatitis E Virus. Vopr Virusol. 1994. 39(4): 165-168.
20. Maneerat Y, Clayson ET, Myint KS, Young GD, Innis BL. Experimental infection of the laboratory rat with the Hepatitis E Virus. J Med Virol. 1996. 48(2): 121-128.
21. Meng XJ, Halbur PG, Shapiro MS, Govindarajan S, Bruna JD, Mushahwar IK, Purcell RH, Emerson SU. Genetic and experimental evidence for cross-species infection by swine Hepatitis E Virus. J Virol. 1998. 72(12): 9714-9721.
22. Anderson DA, Li F, Riddell M, Howard T, Seow H-F, Torresi J, Perry G, Sumarisidi D, Shrestha S M, Shrestha IL. ELISA for IgG-class antibody to Hepatitis E Virus based on a highly conserved, conformational epitope expressed in *Eschericia coli*. J. of Virol. Methods. 1999. 81: 131-142.
23. Chen HY, Lu Y, Howard T, Anderson D, Fong PY, Hu WP, Chia CP, Guan M. An Immunochromatographic Test and its comparison to Enzyme-linked Immunosorbent assay for rapid detection of Immunoglobulin M antibodies to Hepatitis E Virus in patient sera. Clin Diagn Lab Immunol. 2005, 12: 593-598.
24. Hu WP, Lu Y, Precioso NA, Chen HY, Howard T, Anderson D, Guan M. Double-antigen Enzyme-linked Immunosorbent assay for detection of Hepatitis E Virus-specific antibodies in human or swine sera. Clin Vaccine Immunol. 2008. 15(8): 1151-1157.

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