



HEV IgM ELISA 3.0

Instructions For Use

**FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES**

REVISION DATE: 08/09 **Note: Changes Highlighted**
MBQ 0012-ENG-1

REF 23162-096: (96 tests)

NAME AND INTENDED USE

The **MPD DIAGNOSTICS (MPD) HEV IgM ELISA 3.0** is an enzyme-linked immunosorbent assay intended for the detection of IgM antibodies to Hepatitis E Virus (HEV) in human serum or plasma.

This kit is supplied for research use only. It is not intended for use as in the diagnosis or prognosis of disease.

INTRODUCTION

Hepatitis E is known as enterically transmitted non-A non-B hepatitis (ET-NANB) and the etiology agent for this disease has been well established as an non-enveloped, positive sense, single stranded RNA virus named as hepatitis E virus (HEV) (1-3). Although the disease is self-limiting with a mortality rate of 1 to 3% in general adult populations, hepatitis E infection in pregnant women can take more severe forms, with a case fatality rate up to 20%, especially during the third trimester (4). Increasing evidence suggests that this hepatitis occurs not only in developing areas such as Central and South Asia, North and West Africa, Middle East and in Mexico, but also in industrialized nations and areas including United States, Japan and Europe. Hence, the disease might be more widespread than previously recognized (5-6). The recent outbreaks of HEV in Chad and Sudan provide a reminder of this concern. Over a 4-month period, 6861 suspected hepatitis E cases with 87 deaths occurred in Sudan and 1442 cases with 46 deaths occurred in Chad, with the highest incidence in over-crowded refugee camps (7-8). In this respect, hepatitis E infection is increasingly an important public concern of global significance (6).

The **MPD HEV IgM ELISA 3.0** is an indirect immunoassay that utilizes a highly conserved conformational epitope (9) derived from open reading frame 2 (ORF 2) of the virus. The presence of IgM antibodies in the specimen is detected by monoclonal mouse anti-human IgM antibodies labelled with horseradish peroxidase.

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on **MPD Diagnostics'** products and packaging. These symbols are the most common ones appearing on medical devices and their packaging. Some of the common symbols are explained in more detail in **European Standard EN 980:2008**.

- Use by
- Batch Code Synonyms for this: Lot Number Batch Number
- Temperature Limitation
- Sufficient for <-> tests
- Do not reuse
- Manufacturer
- Catalogue Number Synonyms for this: Reference Number Re-order Number
- Caution
- Consult Instructions for Use

CHEMICAL AND BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The wells of the polystyrene microplate strips are coated with highly conserved conformational ORF2 epitope. Human serum or plasma, diluted in diluent buffer, are incubated in these coated wells. HEV specific antibodies, if present, will bind to the antigens immobilized on the solid phase. After incubation, the wells are thoroughly washed to remove unbound materials and mouse monoclonal anti-human IgM antibodies labeled with horseradish peroxidase is added to the wells. This labeled antibody will bind to any antigen-antibody complexes previously formed and excess unbound labeled antibodies are removed by washing. A colourless 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 a blue colour after incubation, which changes to yellow when the colour reaction is terminated by the addition of acid. The intensity of the resulting yellow product is measured at 450nm using a spectrophotometer and is proportional to the amount of antibodies present in the specimen.

KIT COMPONENTS

Component Description	Quantity Provided
MICROPLATE	
HEV MICROPLATE	1 Plate (96 tests)
Twelve 8-well strips per plate, sealed in an aluminum pouch with desiccant. Each microplate well contains adsorbed HEV recombinant proteins.	
Content: 96 wells per plate	
Storage: 2°C to 8°C	
CONTROL	1 vial
NON-REACTIVE CONTROL	
Inactivated normal human serum, non-reactive for anti-HCV, anti-HIV 1/2, anti-HEV and HBsAg. Contains thimerosal and sodium azide as preservatives.	
Content: 160µl per vial	
Storage: 2°C to 8°C	

CONTROL

REACTIVE CONTROL 1 vial
Inactivated human serum containing a high titer of IgM antibodies specific for HEV. Contains thimerosal and sodium azide as preservatives.
Content: 80µl per vial
Storage: 2°C to 8°C

DILUENT

SAM DILUENT 1 bottle
(SAM = Sample Addition Monitor)
Tris based saline solution containing heat-treated normal goat serum, bovine serum albumin and stabilizers. Contains Bronidox™ as preservative.
Content: 100ml per bottle
Storage: 2°C to 8°C

WASH/PLATE 20x

PLATE WASH CONCENTRATE (20x) 1 bottle
Phosphate buffered saline with Tween-20. Contains chloroacetamide as preservative.
Content: 120ml per bottle
Storage: 2°C to 8°C

CONJUGATE

CONJUGATE 1 vial
Mouse monoclonal anti-human IgM labelled with horseradish peroxidase. Contains 0.02% thimerosal as preservative.
Content: 70µl per vial
Storage: 2°C to 8°C

SUBSTRATE

SUBSTRATE 1 bottle
Colourless solution containing 3,3',5,5'-tetramethylbenzidine (TMB).
Content: 12.5ml per bottle
Storage: 2°C to 8°C in the dark

STOP SOLUTION

STOP SOLUTION 1 bottle
1 N hydrochloric acid solution
Content: 30ml per bottle
Storage: 2°C to 8°C in the dark

PLATE COVERS

Adhesive covers for microplate during incubation. 4 pieces

INSTRUCTIONS FOR USE

Bronidox™ is a Trade Mark of Henkel Chemical Co.

WARNINGS AND PRECAUTIONS

- For Research Use only.
- 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, 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.
R52 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).

- Avoid microbial contamination of reagents when opening and removing aliquots from the original vials or bottles.
- Do not pipette by mouth.

- Handle assay specimens, microplates, Reactive and Non-Reactive Controls as potentially infectious agents.
- Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in biohazard waste-bags. Wash hands thoroughly afterwards.
- It is highly recommended that this assay be performed in a biohazard cabinet.
- Keep materials away from food and drink.
- In case of an accident or contact with eyes, rinse immediately with plenty of water and seek medical advice.
- 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.
- Do not expose substrate to strong light.
- Wipe spills of potentially infectious materials immediately with absorbent paper and swab the contaminated area with an effective disinfecting agent before work is resumed.
- Autoclave all used and contaminated materials at 121°C, 15 psi for 30 minutes before disposal.
- Decontaminate all used chemicals and reagents by adding sufficient volume of effective disinfecting agent and leave for 30 minutes.

ANALYTICAL PRECAUTIONS

- Use only serum or plasma samples. Before storage, ensure that the blood clot or blood cells have been separated by centrifugation.
- Do not use whole blood or bodily fluids.
- Optimal assay performance requires **STRICT ADHERENCE** to the assay procedure described in this instruction Manual. Deviations from the procedure may lead to aberrant results.
- 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.
- Do not use kit components beyond the expiry date printed on the kit box.
- 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.
- 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.
- 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.
- For best results allow all reagents and samples to reach room temperature (25°C ± 5°C) before use. Immediately after use return to 2°C to 8°C storage.
- Use only reagent grade quality, deionised or distilled water to dilute reagents.

- ALL REAGENTS MUST BE MIXED WELL BEFORE USE.**
- WORKING CONJUGATE SOLUTION SHOULD BE PREPARED FRESH PRIOR TO USE.**
- 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.
- 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.
- The kit controls should be assayed concurrently with samples for each test run.
- 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.
- Use of highly haemolyzed samples, incomplete clotted sera, plasma samples containing fibrin or samples with microbial contamination may give rise to erroneous results.
- DO NOT USE A WATER BATH TO INCUBATE MICROPLATES.**
- During 37°C incubation, evaporation must be prevented. Cover plates with adhesive covers provided.
- Avoid repeatedly opening and closing the incubator door during incubation steps.
- 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.
- Ensure that automated equipment if used is validated before use.
- Routine maintenance of aspiration / wash system is strongly recommended to prevent carryover from highly reactive specimens to non-reactive specimens.

STORAGE

- Store **MPD HEV IgM ELISA 3.0** kit and its components at 2°C to 8°C when not in use.
- All test reagents and microplates when stored at 2°C to 8°C, are stable until the expiry date given on the kit. Do not freeze the reagents.
- 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.
- Opened, unused microplate strips must be stored with the desiccant provided at 2°C to 8°C in a closed pouch.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Specimen should be stored 2°C to 8°C if the test is to be run within 7 days of collection or frozen at 20°C or colder if the test is to be delayed for more than 7 days.

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

Sera can be inactivated but this is not a requirement for optimal test performance. If necessary, inactivate as follows:
1. Loosen caps of serum containers.
2. Heat serum to 56°C for 30 minutes in a water bath.
3. Allow serum to cool before retightening caps.
4. Serum can be stored frozen until analysis. Repeated freeze-thawing of the sample is not recommended.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Disposable absorbent benchtop paper and paper towels.
- Polypropylene tubes or containers.
- Graduated pipettes: 5ml, 10ml.
- Multichannel pipettor capable of delivering 50µl, 100µl, and 200 µl.
- Pipettor capable of delivering 1-1000µl.
- Disposable pipette tips.
- Reagent reservoirs (troughs) with a capacity of 25ml.
- Deionised or distilled water, reagent grade quality.
- Flasks: 500ml, 1 litre.
- An ELISA Microplate Washer. Alternatively, washing can be performed manually by using a multichannel pipettor delivering 0.3ml volumes and an aspirator device.
- A 37°C incubator.
- A dual (A₅₅₀ - A₆₂₀) or single (A₄₅₀) wavelength microassay plate reader.
- Sodium hypochlorite (5%) solution or liquid household bleach.

PREPARATION OF REAGENTS

- WORKING CONJUGATE**
a. **WORKING CONJUGATE** should be prepared fresh prior to use.
b. Mix **CONJUGATE** and **DILUENT** thoroughly before use. **DO NOT SPIN** the conjugate vial.
c. Dilute **CONJUGATE** at 1200 dilution factor with **SAM DILUENT**. For example, add 10µl conjugate into 2.0ml diluent.
d. Use only polypropylene containers or tubes.
e. 12.0 ml of **WORKING CONJUGATE** is required for one microplate.

CONJUGATE PREPARATION CHART (1:200 dilution factor)		
Number of tests	Vol. of Conjugate (µl)	Vol. of SAM Diluent (ml)
24	20	4
48	30	6
72	45	9
96	60	12

- DILUTED PLATE WASH (1x PLATE WASH)**
a. **DILUTED PLATE WASH (1x PLATE WASH)** is stable for 2 weeks at room temperature.
b. Dilute 1 volume of **20x PLATE WASH** 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.

- Equilibrate all kit components and test specimens to room temperature before use. Remove microplate from the aluminium bag. Mix specimen and control vials thoroughly before use.
- Fill a reagent reservoir with **SAM DILUENT**. Using a multichannel pipettor, add **200µl** of **SAM DILUENT** to all wells.
- Wells A1 and B1 are **'BLANKS' DO NOT ADD SPECIMEN TO THESE WELLS.**
- Add **10µl** of specimen to the assigned well, starting at well H1. This will give a final specimen dilution of 1: 21. **Mix by pipetting up and down at least once.** Repeat this step with other specimens until all are added. **10µl**
- Add **10µl** of **NON-REACTIVE CONTROL** per well to wells C1, D1 & E1. **Mix by pipetting up and down at least once.** **10µl**
- Add **10µl** of **REACTIVE CONTROL** per well to wells F1 and G1. **Mix by pipetting up and down at least once.** **10µl**
- Tap gently on all sides of the microplate to ensure proper mixing of the specimens and controls. Carefully cover the microplate with a plate cover provided to prevent evaporation during incubation.
- Incubate for 30 minutes at 37±1°C. (Do not use a water bath for incubation).** **30±2 min 37±1°C**
- Prepare **WORKING CONJUGATE** as described in the **PREPARATION OF REAGENTS** prior to washing the microplate.
- Remove and discard the plate cover and wash the microplate with **DILUTED PLATE WASH (1x PLATE WASH)** using one of the two recommended methods. **300µl per well x 6**
- Automated or Semi-automatic Microplate Washer - Wash six (6) times with at least 300µl per well per wash.
- 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/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 the **WORKING CONJUGATE.** Using a multichannel pipettor, add 100µl of **WORKING CONJUGATE** to each well. Apply another plate cover.

14. **Incubate the microplate for 30 minutes at 37±1° C (Do Not use a water bath for incubation).**

15. Remove and discard the plate cover. Repeat the wash procedure as in Step 11 and Step 12.

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

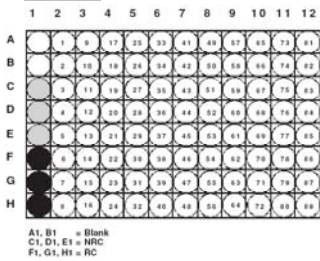
17. Incubate for 15 minutes in the dark at room temperature (25±3° C).

18. Remove and discard the plate cover.

19. Using a multichannel pipettor, add 100µl of **STOP SOLUTION** to each well. Mix gently by tapping the plate.

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

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



QUALITY CONTROL

1. Please ensure that each specimen and control is properly mixed with **SAM DILUENT** by pipetting up and down at least once after addition.
2. Change in colour of **SAM DILUENT** indicates that serum or plasma has been added.
3. The **BLANK** and the **REACTIVE CONTROL** should be assayed in duplicate, whereas the **NON REACTIVE CONTROL** in triplicate on each plate with each run of specimens.
4. Blank values must have an absorbance of ≤ 0.100 .
5. Non-Reactive Control values must have an absorbance ≤ 0.100 after subtracting the Blank.
6. Both **REACTIVE CONTROL** values must have absorbance ≥ 0.500 after subtracting the Blank.
7. For invalid assays, refer to **TROUBLESHOOTING GUIDE**.

ASSAY CUT-OFF VALUE

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 IgM antibodies specific for HEV is determined by relating the absorbance of the specimens to the CUT-OFF Value (COV) of the assay. The CUT-OFF Value is calculated as $(0.400 + \text{NRC Mean Absorbance}) \times$

CUT-OFF Value (COV) = 0.400 + NRCx

CALCULATIONS OF RESULTS

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

Example:	Well No.	Absorbance
	C1	0.010
	D1	0.012
	E1	0.008
	Total	0.030
	Mean	$0.030/3 = 0.010$ (NRCx)

Individual Non-Reactive Control values should be ≤ 0.100 . If one Non-Reactive Control value does not meet either of the above criteria, it must be excluded as aberrant. The Non-Reactive Control Mean (NRCx) 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 (RCx)

Example:	Well No.	Absorbance
	F1	0.758
	G1	0.732
	Total	1.490
	Mean	$1.490/2 = 0.745$ (RCx)

Individual Reactive Control must be ≥ 0.500 . If one Reactive Control value does not meet both of the above criteria, the assay is invalid and must be repeated.

3. Calculation of CUT-OFF Value (COV)

CUT-OFF VALUE = 0.400 + NRCx

Example: NRCx = 0.010
CUT-OFF Value = $0.400 + 0.010$
= 0.410

INTERPRETATION OF RESULTS

1. Specimens with absorbance values **less** than the CUT-OFF value are considered **Non-Reactive** by the **MPD HEV IgM ELISA 3.0**.
2. Specimens with absorbance values **greater than or equal** to the CUT-OFF value are considered **Initially reactive** by the criteria of the **MPD HEV IgM ELISA 3.0** and should be retested in duplicate before interpretation.
3. Specimens found reactive on retesting are to be interpreted as **repeatedly reactive** for antibodies to HEV by the criteria of the **MPD HEV IgM ELISA 3.0**.
4. Initially reactive specimens which are **Non-Reactive** on retesting are considered **negative** by the criteria of the **MPD HEV IgM ELISA 3.0**.

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance of **MPD HEV IgM ELISA 3.0** was evaluated with over 2000 blood samples, among them was a population of other hepatitis patients (n=665) who had similar clinical presentations as hepatitis E. The **MPD HEV IgM ELISA 3.0** was able to maintain a specificity of 95.8% within this group of patient and at the same time detected the acute hepatitis E patients (n=903) with a sensitivity of 98%. Furthermore, it was able to maintain a background specificity of 97.6% for healthy population (n=777). This gave **MPD HEV IgM ELISA 3.0** a positive predictive value of 94.9% and a negative predictive value of 98.7% (Table 4).

For study one (table 1) (10), samples from patients with acute hepatitis were obtained from Nepal and China. Samples from patients with other hepatitis or other infections were either collected locally in Australia or purchased from a commercial source. The overall sensitivity and specificity for this study were 99.3% and 97.6% respectively.

For the second study (Table 2), the patient samples with HEV infections were from Indonesia and Nepal. Samples from patients with other hepatitis or healthy controls were from Thailand, Nepal, Cambodia and Indonesia. The overall sensitivity and specificity for this study were 99.5% and 89.8% respectively.

From a two-site clinical trial in China (Table 3), the sensitivity and specificity of the combined study is 97.0% and 97.8% respectively.

From a prospective clinical trial from China, the HEV detection rate of an acute hepatitis population of non-A, non-B and non-C origin (n=153) is 32.0%. **MPD HEV IgM ELISA 3.0** detected 49/50 cases of confirmed HEV patient

from this population for a sensitivity of 98.0% with an accompanying specificity of 100.0% (103/103). The specificity for hepatitis B (n=25), hepatitis C (n=25) and healthy donors (n=148) at this site are all 100.0%.

Table 1:

Serum group and patient status	No. of Positives & Performance %
Serum from patients with:	
Acute hepatitis E (Nepal, outbreak)	79/80
Acute hepatitis E (Nepal, sporadic)	30/30
Acute hepatitis E (Nepal, sporadic)	41/41
Total Sensitivity	99.3% (n=151)
From other hepatitis patients:	
HAV antibody positive	2/65
HBSAg positive	1/13
HCV antibody positive	0/10
From other patients controls:	
HSV IgM positive	0/10
Toxo IgM positive	0/5
Rheumatoid Arthritis	1/5
Systemic Lupus Erythematosus	0/5
Healthy controls:	
Blood donors (Australia)	0/30
Healthy individuals (Nepal)	1/30
Blood donors (USA)	0/35
Total Specificity	97.6% (n=208)

Table 2:

Serum group and patient status	No. of Positives & Performance %
Sera from patients with:	
Acute hepatitis E (PCR and IgM positive)	199/200
Total Sensitivity	99.5% (n=200)
From other hepatitis patients:	
Acute hepatitis A	8/80
Acute hepatitis B	1/45
Acute hepatitis C	8/50
Healthy donors (Thailand)	11/200
Total Specificity	89.8% (n=275)

Table 3:

Serum group and patient status	No. of Positives & Performance %
Sera from patients with:	
Clinically diagnosed acute hepatitis E	487/502
Total Sensitivity	97.0% (n=502)
From other hepatitis patients:	
Hepatitis A	3/62
Hepatitis B	1/24
Hepatitis C	4/63
Healthy donors	7/434
Total Specificity	97.8% (n=683)

Table 4: Summarized Data (inclusive of the prospective clinical trial from China)

Serum group and patient status	No. of Positives & Performance %
Sera from patients with acute hepatitis E	855/805
Total Sensitivity	98.0% (n=805)
From other non hepatitis patients	1/25
From other hepatitis patients	28/665
Healthy donors	19/777
Total Specificity	96.7% (n=1467)
Positive Predictive Value (PPV)	94.5%
Negative Predictive Value (NPV)	98.7%

Reproducibility

The assay precision of the **MPD HEV IgM ELISA 3.0** was evaluated in-house using three serum calibrators including a positive, a weak positive and a negative serum.

Within-run: Three kits of different lot numbers (comprising entirely three different sets of ELISA components) were assayed as 30 replicates per serum calibrator on 3 occasions. The coefficient of variation (CV) for the 3 calibrators in different run varied between 3.5% and 11.9% (Table 5).

Between-run: A total of 90 observations were recorded to assess between-run precision. These observations represent 3 runs using 3 lots of ELISA components with each serum calibrator. The coefficient of variation for the 3 calibrators varied between 5.4% and 12.6% (Table 5).

Total precision: This is carried out using different kits (comprising entirely three different sets), a 5-member positive panel and a 5-member negative panel to give 10 calibrators. Three lots of ELISA components were assayed on each occasion. Each kit was repeatedly tested with the 10 calibrators for 5 times over a period of 20 days by 3 operators. The overall precision was assessed with 150 normalized data points (OD/COV) obtained with each calibrator. The coefficient of variation is 15.6%.

Samples	Kit Lot No.	No. of Replicates	Mean OD/OV	Within-run Precision (CV, %)	Between-run Precision (CV, %)
Positive	1	30	2.856	4.5	5.4
	2	30	2.732	3.7	
	3	30	2.617	4.0	
Weak Positive	1	30	1.169	9.2	12.6
	2	30	0.991	6.1	
	3	30	0.970	11.9	
Negative	1	30	0.381	6.8	6.3
	2	30	0.355	3.5	
	3	30	0.350	4.4	

TROUBLESHOOTING GUIDE

Problem	Possible Causes	Solution
Invalid assay due to out-of-specification absorbance value of Blank or NRC (<0.100)	<ul style="list-style-type: none"> Incubation temperature out of range Incorrect dispensing volume Incubation time longer than specified Incorrect dilution for Working Conjugate Cross-contamination of controls Control and Working Conjugate not well mixed Component mixed-up from different kits Insufficient washing 	<ul style="list-style-type: none"> Check preparation of reagents, ensure reagents are well mixed before use Check control for signs of contamination Do not use components from different lot of kits Verify calibration and maintenance program for pipettor, multichannel pipettor, incubator, microplate washer and reader
Invalid assay due to out-of-specification absorbance value of RC (<0.500), or overall weak or completely no color development	<ul style="list-style-type: none"> Incubation temperature out of range Incorrect dispensing volume Insufficient incubation time Incorrect dilution for Working Conjugate Control and Working Conjugate not well mixed Controls and test specimens not equilibrated to room temperature before assay Deterioration in enzymatic activity of conjugate Deterioration of microplate Incorrect reading filter or interference of optical pathway Component mixed-up from different kits Excessive washing 	<ul style="list-style-type: none"> Check preparation of reagents, ensure reagents are well mixed before use Do not spin conjugate vial before use Check control for signs of contamination Check expiry date of kit and its components Do not use components from different lot of kits Equilibrate reagents to room temperature before use Verify calibration and maintenance program for pipettor, multichannel pipettor, incubator, microplate washer and reader Use clean containers and troughs Check the quality of distilled or deionized water used for dilution Verify calibration and maintenance program for pipettor, multichannel pipettor, incubator, microplate washer and reader Check that 1X Plate Wash is within 2 weeks shelf-life after preparation
Valid assay but overall color development is too strong with too many initial reactive specimens	<ul style="list-style-type: none"> Incubation temperature out of range Incorrect dispensing volume Incubation time longer than specified Overdilatation of substrate due to improper storage or use of dirty trough Wrong preparation of 1X Wash Buffer Deterioration of 1X Plate Wash Insufficient or ineffective washing 	<ul style="list-style-type: none"> Check preparation of reagents, ensure reagents are well mixed before use Check control for signs of contamination Check expiry date of kit and its components Do not use components from different lot of kits Equilibrate reagents to room temperature before use Use clean containers and troughs Check the quality of distilled or deionized water used for dilution Verify calibration and maintenance program for pipettor, multichannel pipettor, incubator, microplate washer and reader Check that 1X Plate Wash is within 2 weeks shelf-life after preparation

LIMITATIONS OF THE PROCEDURE

Deviation from the recommended procedure may lead to aberrant results.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no expressed warranty other than that the test kit will function as a Research Use Only assay within the specifications and limitations described in the product Instruction Manual when used in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied, including such expressed 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 whatsoever caused by the product in the use or in the application thereof.

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.

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- * US Patent 5,741,490; 5,770,689; 5,885,768; 5,686,239; 6,514,690B1
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