HEV ELISA 4.0

Instructions for Use

CE

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Note Changes Highlighted

REF 23540-096: (96 tests)

NAME AND INTENDED USE

The **MP Diagnostics 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. It is intended as a screening test, requiring repeat testing of initially reactive specimens.

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 **MP Diagnostics 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. Some of the common symbols are explained in more detail in the European and International Standard EN ISO 15223: 2012.

	Use by	IVD	*In vitro* diagnostic medical device
LOT	Batch Code	REF	Catalogue Number
	Temperature Limitation		Caution
	Manufacturer	EC REP	Authorized Representative in the European Community
Σ	Sufficient for <n> tests		Consult Instructions for Use
	Do not reuse		
CONT	Contents		

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 in vitro diagnostic use only.
2. For Professional use only.
3. Please refer to the product labelling 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.

Pursuant to EC regulation 1272/2008 (CLP), hazardous components are classified and labelled as follows:

Component:	**Plate Wash Concentrate (20x)**
Signal Word:	**Warning**
Pictogram:	
Hazard Statements:	H317 May cause an allergic skin reaction
Precautionary Statements:	P261 Avoid breathing dust/ fume/gas/mist/vapours/ spray. P272 Contaminated work clothing should not be allowed out of the workplace. P302+P352 IF ON SKIN: Wash with plenty of soap and water. P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
Supplemental Statements:	EUH210 Safety Data Sheet is available on request
Contains:	2% Chloroacetamide

Component:	**Stop solution**
Signal Word:	**Danger**
Pictogram:	
Hazard Statements:	H315 Causes skin irritation. H319 Causes serious eye irritation.

Precautionary Statements:	P264 Wash hands thoroughly after handling. P280 Wear protective gloves/protective clothing/ eye protection/face protection. P312 Call a POISON CENTER or doctor/physician if you feel unwell. P362 Take off contaminated clothing and wash before reuse. P302+P352 IF ON SKIN: Wash with plenty of soap and water. P332+P313 If skin irritation occurs: Get medical advice/ attention. P337+P313 If eye irritation persists: Get medical advice/ attention. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Supplemental Statements:	EUH210 Safety Data Sheet is available on request
Contains:	11.2% Sulphuric acid

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 **MP Diagnostics 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

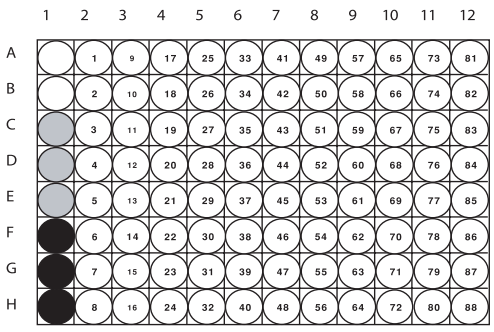
14. Incubate for 30 minutes in the dark at 37°C. **(Do Not use a 37°C water bath for incubation).** — 30 min

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.



A1, B1 = Blank
C1, D1, E1 = NRC
F1, G1, H1 = RC

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 ($RC\bar{x}$).

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):

$$\text{CUT-OFF VALUE} = 0.40 + NRC\bar{x}$$

CALCULATION OF RESULTS

1. Calculation of Non-Reactive Control Mean Absorbance ($NRC\bar{x}$)

Example:	Well No.	Absorbance
	C1	0.050
	D1	0.051
	E1	0.052
	Total	0.153
	Mean	0.153 / 3 = 0.051 ($NRC\bar{x}$)

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 ($NRC\bar{x}$) 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 ($RC\bar{x}$)

Example:	Well No.	Absorbance
	F1	1.221
	G1	1.144
	H1	1.298
	Total	3.663
	Mean	3.663 / 3 = 1.221 ($RC\bar{x}$)

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 ($RC\bar{x}$) 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 $RC\bar{x}$ and $NRC\bar{x}$

Example:		
	$NRC\bar{x}$	= 0.051
	$RC\bar{x}$	= 1.221
	$RC\bar{x}$ - $NRC\bar{x}$	= 1.221 - 0.021
		= 1.200

For the assay to be valid, the $RC\bar{x}$ - $NRC\bar{x}$ 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

Example:		
	CUT - OFF Value	= 0.40 + $NRC\bar{x}$
	$NRC\bar{x}$	= 0.051
	CUT - OFF Value	= 0.40 + 0.051
		= 0.451

INTERPRETATION OF RESULTS

1. Specimens with absorbance values less than the CUT - OFF value are considered **Non-Reactive** by the **MP Diagnostics HEV ELISA 4.0**.

2. Specimens with absorbance values **greater than or equal to** the CUT - OFF value are considered **initially reactive** by the criteria of the **MP Diagnostics HEV ELISA 4.0** and should be retested in duplicate before interpretation.

3. Specimens found Reactive on retesting may be interpreted to be **repeatedly reactive** for antibodies to HEV by the criteria of the **MP Diagnostics HEV ELISA 4.0**.

4. Initially reactive specimens which are **Non-Reactive** on retesting are considered **negative** by the criteria of the **MP Diagnostics HEV ELISA 4.0**.

SPECIFIC PERFORMANCE CHARACTERISTICS

Sensitivity
189 HEV IgM positive (acute infection) and 67 HEV IgG positive/ IgM negative (past infection) samples were studied in house. The results summarised in Table 1 showed a sensitivity of 99.5% for 189 confirmed HEV IgM positive samples, and 98.5% for HEV IgG positive but IgM negative samples.

Table 1 Assay Performance – Sensitivity

Sample Type	No. of Samples	Reactive	Negative	Sensitivity
HEV IgM positive (Nepal)	151	150	1	99.3 %
HEV IgM positive (China)	38	38	0	100 %
HEV IgG positive/IgM negative (archived)	67	66	1	98.5 %
Total	256	254	2	99.2 %

Specificity
A total of 368 samples comprising of blood donor samples (n=236) and potentially cross-reactive samples (n=132) were tested. The results, summarised in Table 2, showed a diagnostic overall specificity of 99.2% for both the blood donor and potentially cross-reactive samples.

Table 2 Assay Performance – Specificity

Other Disease Controls	No. of Samples	Negative	Reactive	Specificity
Blood Donors (USA)	236	234	2	99.2 %
HAV antibody positive	33	33	0	100 %
HCV antibody positive	43	43	0	100 %
HBsAg positive	20	20	0	100 %
HSV antibody positive	20	19	1	95 %
Rheumatoid Factor	16	16	0	100 %
Total	368	365	3	99.2 %

Reproducibility
The assay precision of the **MP Diagnostics HEV ELISA 4.0** was evaluated in-house using 2 serum calibrators including a HEV IgG positive sample and a HEV IgM positive sample.

Within-run: Three lots 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 3.7% and 5.7% (Table 3).

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 coefficients of variation for the 2 calibrators varied between 5.8% and 10.8% (Table 3).

Table 3 Assay Performance – Reproducibility

Samples	Assay Components	No. of Replicates	Mean OD/COV	Within-run Precision (CV, %)	Between-run Precision (CV, %)
HEV IgG Positive	#1	30	5.344	3.7	5.8
	#2	30	5.752	4.6	
	#3	30	5.337	5.4	
HEV IgM Positive	#1	30	8.065	5.3	10.8
	#2	30	7.507	4.3	
	#3	30	6.391	5.7	

Total precision: Three lots of ELISA components were assayed as 5 replicates per serum calibrator on each occasion. This is repeated 30 times over a period of 21 days by 4 operators. The overall precision was assessed with 300 data points (OD/COV) obtained with 2 serum calibrators. The coefficients of variation for the 2 calibrators varied between 12.1% and 15.7%.

LIMITATIONS OF THE METHOD

Repeatedly reactive results from the **MP Diagnostics HEV ELISA 4.0** is a presumptive evidence of antibodies to HEV in the specimen. A **NON-REACTIVE** result from the **MP Diagnostics HEV ELISA 4.0** indicates the likely absence of detectable antibodies to HEV in the specimen. A **NEGATIVE** result does not exclude the possibility of exposure to or infection with HEV.

Falsely reactive results can be suspected with a test kit of this nature. The proportions of false reactives will depend on the sensitivity and the specificity of the test kit. For most screening assays, the higher the prevalence of antibody in a population, the lower the proportion of falsely reactive samples.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no express warranty other than that the test kit will function as an *in vitro* diagnostic assay within the 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.

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MP Biomedicals Asia Pacific Pte. Ltd.
2 Pioneer Place
Singapore 627885
Tel No. : + 65 6775 0008
Fax No. : + 65 6774 6146
Email : enquiry_ap@mpbio.com

EC REP
MP Biomedicals Germany GmbH
Thüringer Straße 15
37269 Eschwege
Germany
Tel. No. : +49 5651 921 204
Fax No. : +49 5651 921 181
Email : diagnostics@mpbio.com

Regional Office:

MP Biomedicals Germany GmbH
Thüringer Straße 15
37269 Eschwege
Germany
Tel. No. : +49 5651 921 204
Fax No. : +49 5651 921 181
Email : diagnostics@mpbio.com