



## Instructions for Use

# HEV IgG ELISA



**REF** EIA-4145



96



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**Please use only the valid version of the Instructions for Use provided with the kit.  
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.  
Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.  
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.**

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## 1 INTENDED USE

Third generation Enzyme ImmunoAssay (ELISA) for the qualitative determination of IgG antibodies to Hepatitis E Virus in human plasma and sera.

The kit is intended for the follow-up of HEV-infected patients.

For "in vitro" diagnostic use only.

## 2 INTRODUCTION

Hepatitis E Virus or HEV is a recently discovered agent of enterically transmitted viral hepatitis. HEV is an un-enveloped single-strand RNA virus, after being provisionally assigned to the Caliciviridae family, HEV was re-classified as the sole member of the genus Hepevirus, family Hepeviridae, in 2004.

HEV is found in the stool of infected patients and present in 4 strains (1, 2, 3 and 4) differently spread geographically and virulent.

HEV is a serious problem in many developing countries since its first outbreak was reported in 1955 in New Delhi, India.

A high case-fatality rate has been found among pregnant women and chronic hepatitis carriers.

The cloning and sequencing of HEV genome have led to the development of serological tests for the detection of anti HEV antibodies based on recombinant immunodominant antigens derived from conservative regions of the four virus strains.

## 3 PRINCIPLE OF THE TEST

Microplates are coated with HEV-specific recombinant antigens encoding for conservative and immunodominant determinants of all the 4 subtypes.

The solid phase is first treated with the diluted sample and anti-HEV IgG are captured, if present, by the antigens.

After washing out all the other components of the sample, in the 2<sup>nd</sup> incubation bound anti-HEV IgG are detected by the addition of polyclonal specific anti-hIgG antibodies, labelled with peroxidase (HRP).

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HEV IgG present in the sample.

A cut-off value let optical densities be interpreted into anti-HEV IgG negative and positive results.

#### 4 COMPONENTS

EIA-4145 contains reagents for 96 tests.

1. **Microplate** MICROPLATE  
 n°1 microplate.  
 12 strips of 8 microwells coated with HEV specific recombinant antigens  
 Plates are sealed into a bag with desiccant.
2. **Negative Control** CONTROL -  
 1 x 4.0 mL/vial. Ready to use control.  
 It contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 6.0 ± 0.1, 0.5% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.  
 The negative control is olive green colour coded.
3. **Positive Control** CONTROL +  
 1 x 4.0 mL/vial. Ready to use control.  
 It contains 1% goat serum proteins, human antibodies positive to HEV, 10 mM Na-citrate buffer pH 6.0 ± 0.1, 0.5% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.  
 The positive control is blue colour coded.
4. **Calibrator** CAL ...  
 n°1 vial. Lyophilized calibrator.  
 To be dissolved with the volume of EIA grade water reported on the label.  
 It contains foetal bovine serum proteins, human antibodies to HEV whose content is *calibrated on 1<sup>st</sup> WHO reference reagent for HEV antibody, NIBSC code 95/584 at 1 IU/mL ± 20 %*,  
 10 mM Na-citrate buffer pH 6.0 ± 0.1, 0.3 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.  
**Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.**
5. **Wash buffer concentrate** WASHBUF 20X  
 1 x 60 mL/bottle. 20x concentrated solution.  
 Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0 ± 0.2, 0.05% Tween 20 and 0.1% Kathon GC.
6. **Enzyme Conjugate** CONJ  
 1 x 16 mL/vial. Ready to use and red colour coded reagent.  
 It contains Horseradish Peroxidase conjugated goat polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8 ± 0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.
7. **Chromogen/Substrate** SUBS TMB  
 1 x 16 mL/vial. Ready-to-use component.  
 It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H<sub>2</sub>O<sub>2</sub>.  
**Note: To be stored protected from light as sensitive to strong illumination.**
8. **Assay Diluent** DILAS  
 1 x 8 mL/vial.  
 10 mM tris buffered solution pH 8.0 ± 0.1 containing 0.1% Kathon GC  
for the pre-treatment of samples and controls in the plate, blocking interference.
9. **Sulphuric Acid** H2SO4 0.3 M  
 1 x 15 mL/vial.  
 It contains 0.3 M H<sub>2</sub>SO<sub>4</sub> solution.  
 Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363)
10. **Sample Diluent:** DILSPE  
 1 x 50 mL/vial.  
 It contains 10 mM Na-citrate buffer pH 6.0 ± 0.1, 0.5% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.  
To be used to dilute the sample.  
**Note: The diluent changes colour from olive green to dark bluish green in the presence of sample.**
11. Plate sealing foils n°2
12. Package insert n°1

**5 MATERIALS REQUIRED BUT NOT PROVIDED**

1. Calibrated Micropipettes (200 µL and 10 µL) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator capable to provide a temperature of +37 °C.
6. Calibrated ELISA microwell reader with 450 nm (reading) and with 620-630 nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

**6 WARNINGS AND PRECAUTIONS**

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2 °C - 8 °C into a temperature controlled refrigerator or cold room.
7. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
14. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hours or heat inactivation by autoclave at 121°C for 20 minutes.
15. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
16. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
17. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

## 7 SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2 °C - 8 °C for up to seven days after collection. For longer storage periods, samples can be stored frozen at -20 °C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2 - 0.8 µm filters to clean up the sample for testing.

## 8 PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.

### 1. Microplates:

Allow the microplate to reach room temperature (about 1 hour) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of storing. In this case call the customer service.

Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at 2 °C - 8 °C. When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

### 2. Negative Control:

Ready to use. Mix well on vortex before use.

### 3. Positive Control:

Ready to use. Mix well on vortex before use. Handle this component as potentially infective, even if HCV, eventually present in the control, has been chemically inactivated.

### 4. Calibrator:

Dissolve carefully the content of the lyophilized vial with the volume of EIA grade water reported on its label. Mix well on vortex before use. Handle this component as potentially infective, even if HEV, eventually present in the control, has been chemically inactivated.

**Note:** When dissolved the Calibrator is not stable. Store in aliquots at -20 °C.

### 5. Wash buffer concentrate:

The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 mL and mixed gently end-over-end before use. Once diluted, the wash solution is stable for 1 week at 2 °C - 8 °C. In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.

**Note:** Once diluted, the wash solution is stable for 1 week at 2 °C - 8 °C

### 6. Enzyme conjugate:

Ready to use. Mix well on vortex before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes. If this component has to be transferred use only plastic, possibly sterile disposable containers.

**7. Chromogen/Substrate:**

Ready to use. Mix well on vortex before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong illumination, oxidizing agents and metallic surfaces. If this component has to be transferred use only plastic, possible sterile disposable container.

**8. Assay Diluent:**

Ready to use. Mix well on vortex before use.

**9. Sulphuric Acid:**

Ready to use. Mix well on vortex before use.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363)

Legend:**Warning H statements:**

**H315** – Causes skin irritation.

**H319** – Causes serious eye irritation.

**Precautionary P statements:**

**P280** – Wear protective gloves/protective clothing/eye protection/face protection.

**P302 + P352** – IF ON SKIN: Wash with plenty of soap and water.

**P332 + P313** – If skin irritation occurs: 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.

**P337 + P313** – If eye irritation persists: Get medical advice/attention.

**P362 + P363** – Take off contaminated clothing and wash it before reuse.

**10. Sample Diluent:**

Ready to use. Mix well on vortex before use.

**9 INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT**

1. **Micropipettes** have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of  $\pm 2\%$ . Decontamination of spills or residues of kit components should also be carried out regularly.
2. The **ELISA incubator** has to be set at  $+37\text{ }^{\circ}\text{C}$  (tolerance of  $\pm 0.5\text{ }^{\circ}\text{C}$ ) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of  $350\text{ }\mu\text{L}$ /well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20 - 30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. **Incubation times** have a tolerance of  $+ 5\%$ .
5. The **ELISA reader** has to be equipped with a reading filter of 450 nm and with a second filter (620-630 nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth  $\leq 10\text{ nm}$ ; (b) absorbance range from 0 to  $\geq 2.0$ ; (c) linearity to  $\geq 2.0$ ; repeatability  $\geq 1\%$ . Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.

6. When using an **ELISA automated work station**, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended for blood screening when the number of samples to be tested exceed 20-30 units per run.



## 10 PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colourless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above.
5. Allow all the other components to reach room temperature (about 1 hour) and then mix as described.
6. Set the ELISA incubator at +37 °C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

## 11 ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

### 11.1 Automated assay

In case the test is carried out automatically with an ELISA workstation, we suggest to make the instrument aspirate 200 µL Sample Diluent and then 10 µL sample.

All the mixture is then carefully dispensed directly into the appropriate sample well of the microplate. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples.

Do not dilute controls/calibrator as they are ready to use.

Dispense 200 µL controls/calibrator in the appropriate control/calibration wells.

**Important Note:** *Visually monitor that samples have been diluted and dispensed into appropriate wells. This is simply achieved by checking that the colour of dispensed samples has turned to dark bluish-green while the colour of the negative control has remained olive green.*

For the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

**11.2 Manual assay**

1. Place the required number of Microwells in the microwell holder. Leave the first well empty for the operation of blanking.
2. Dispense 200 µL of Negative Control in triplicate, 200 µL Calibrator in duplicate and 200 µL Positive Control in single in proper wells.  
Do not dilute Controls and Calibrator as they are pre-diluted, ready to use !
3. Add 200 µL of Sample Diluent (DILSPE) to all the sample wells; then dispense 10 µL sample in each properly identified well.  
Mix gently the plate, avoiding overflowing and contaminating adjacent wells, in order to fully disperse the sample into its diluent.

**Important note:** Check that the colour of the Sample Diluent, upon addition of the sample, changes from light green to dark bluish green, monitoring that the sample has been really added.

4. Dispense 50 µL Assay Diluent (DILAS) into all the controls/calibrator and sample wells.  
Check that the colour of samples has turned to dark blue.
5. Incubate the microplate for **45 min at +37 °C**.

**Important note:** Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.

6. Wash the microplate with an automatic washer by delivering and aspirating 300 µL/well of diluted washing solution as reported previously (section 9.3).
7. Pipette 100 µL Enzyme Conjugate into each well, except the first blanking well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

**Important note:** Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.

8. Incubate the microplate for **45 min at +37 °C**.
9. Wash microwells as in step 6.
10. Pipette 100 µL Chromogen/Substrate mixture into each well, the blank well included.  
Then incubate the microplate at **room temperature (18 °C - 24 °C) for 15 minutes**.

**Important note:** Do not expose to strong direct illumination. High background might be generated.

11. Pipette 100 µL Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to stop the enzymatic reaction. Addition of acid will turn the positive control and positive samples from blue to yellow.
12. Measure the colour intensity of the solution in each well, as described in section 9.5, at 450 nm filter (reading) and at 620-630 nm (background subtraction, strongly recommended), blanking the instrument on A1.

**Important notes:**

1. If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450 nm. Finger prints could generate false positive results on reading.
2. Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.

**12 ASSAY SCHEME**

Method	Operations
Controls & Calibrator(*)	200 µL
Samples	200 µL dil.+10 µL
Assay Diluent (DILAS)	50 µL
<b>1<sup>st</sup> incubation</b>	<b>45 min</b>
Temperature	+37 °C
Wash step	4-5 cycles
Enzyme conjugate	100 µL
<b>2<sup>nd</sup> incubation</b>	<b>45 min</b>
Temperature	+37 °C
Wash step	4-5 cycles
TMB/H <sub>2</sub> O <sub>2</sub>	100 µL
<b>3<sup>rd</sup> incubation</b>	<b>15 min</b>
Temperature	room temp.
Sulphuric Acid	100 µL
Reading OD	450 nm

**(\*) Important Notes:**

- The Calibrator (CAL) does not affect the Cut Off calculation, therefore it does not affect the test's results calculation.
- The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL(*)	S6										
F	CAL (*)	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank, NC = Negative Control, CAL = Calibrator, PC = Positive Control, S = Sample

### 13 INTERNAL QUALITY CONTROL

A check is carried out on the controls any time the kit is used in order to verify whether their OD<sub>450 nm</sub> values are as expected and reported in the table below.

Check	Requirements
Blank well	< 0.100 OD <sub>450 nm</sub> value
Negative Control (NC)	< 0.050 mean OD <sub>450 nm</sub> value after blanking
Positive Control	> 1.000 OD <sub>450 nm</sub> value

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Problem	Check
<b>Blank well</b> > 0.100 OD <sub>450 nm</sub>	1. that the Chromogen/Substrate solution has not got contaminated during the assay
<b>Negative Control (NC)</b> > 0.050 OD <sub>450 nm</sub> after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control); 4. that no contamination of the negative control or of their wells has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
<b>Positive Control</b> < 1.000 OD <sub>450 nm</sub>	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of positive control. In this case, the negative control will have an OD <sub>450 nm</sub> value > 0.150, too. 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should these problems happen, after checking, report any residual problem to the supervisor for further actions.

#### \*\* Note:

If Calibrator has used, verify the following data:

Check	Requirements
Calibrator	S/Co > 1.1

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
<b>Calibrator</b> S/Co < 2	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of negative control instead of Calibrator) 3. that the washing procedure and the washer settings are as validated in the pre-qualification study; 4. that no external contamination of the calibrator has occurred.

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

## 14 CALCULATION OF THE CUT-OFF

The tests results are calculated by means of a cut-off value determined with the following formula:

$$\text{Cut-Off} = \text{NC mean OD}_{450 \text{ nm}} + 0.350$$

The value found for the test is used for the interpretation of results as described in the next paragraph.

**Important note:** When the calculation of results is done by the operative system of an ELISA automated work station be sure that the proper formulation is used to calculate the cut-off value and generate the right interpretations of results.

## 15 INTERPRETATION OF RESULTS

Test results are interpreted as ratio of the sample OD<sub>450 nm</sub> and the Cut-Off value (or S/Co) according to the following table

S/Co	Interpretation
< 0.9	Negative
0.9 -1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HEV or that the blood unit may be transfused.

Any patient showing an equivocal result should be tested again on a second sample taken 1-2 weeks later from the patient and examined. The blood unit should not be transfused.

A positive result is indicative of HEV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

### Important notes:

1. Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.
2. By definition, any positive result should be confirmed by an alternative method before a diagnosis of viral hepatitis is formulated.
3. When test results are transmitted from the laboratory to an informatics center, attention has to be done to avoid erroneous data transfer.
4. Diagnosis of viral hepatitis infection has to be done and released to the patient only by a qualified medical doctor.

An example of calculation is reported below:

*The following data must not be used instead of real figures obtained by the user.*

Negative Control: 0.019 – 0.020 – 0.021 OD<sub>450 nm</sub>

Mean Value: 0.020 OD<sub>450 nm</sub>

Lower than 0.050 – Accepted

Positive Control: 2.189 OD<sub>450 nm</sub>

Higher than 1.000 – Accepted

$$\text{Cut-Off} = 0.020 + 0.350 = 0.370$$

Calibrator: 0.600 - 0.640 OD<sub>450 nm</sub>

Mean value: 0.620 OD<sub>450 nm</sub> S/Co = 1.7

S/Co higher than 1.1 – Accepted

Sample 1: 0.070 OD<sub>450 nm</sub>

Sample 2: 1.690 OD<sub>450 nm</sub>

Sample 1 S/Co < 0.9 = negative

Sample 2 S/Co > 1.1 = positive

## 16 PERFORMANCES

Evaluation of Performances has been conducted on negative and positive samples in an external clinical center with reference to a FDA approved kit.

### 16.1 Limit of Detection

The limit of detection of the assay has been calculated by means of 1<sup>st</sup> WHO reference reagent for HEV antibody, NIBSC code 95/584.

The assay shows a sensitivity of about 0.1 WHO IU/mL.

### 16.2 Diagnostic Specificity and Sensitivity

They were checked with a sensitivity set at 0.25 WHO IU/mL on more than total 700 samples.

#### 16.2.1 Diagnostic Specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte.

A total number of more than one hundred 1-5 years old children, by definition negative for HEV antibodies as they never chanced to eat uncooked swine meat and get therefore infected, were tested; a value of 100% specificity (negativity) was assured at a sensitivity set at a cut-off of 0.25 WHO IU/mL.

In addition, a total of more 500 unselected donors, including 1<sup>st</sup> time donors, and HEV negative hospitalized patients, coming from Italy, were examined maintaining the sensitivity of 0.25 WHO IU/mL. About 5% of such population turned out to be repeatedly positive; confirmation was not carried out in absence of a commercial Confirmation kit. However, these samples were detected positive with a commercial CE-marked ELISA. From this study a diagnostic specificity of 100% was observed.

Moreover, the Diagnostic Specificity was also assessed by testing more than 100 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, haemolized, lipemic, etc.). A value of specificity of 100% was assessed.

No false reactivity due to the method of specimen preparation has been observed. Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.

Frozen specimens have been tested, as well, to check for interferences due to collection and storage.

No interference was observed.

#### 16.2.2 Diagnostic Sensitivity

It is defined as the probability of the assay of scoring positive in the presence of specific analyte.

The diagnostic sensitivity has been assessed externally (Institute of Virology, University of Milan) and internally on a total number of 200 positive specimens (maintaining a sensitivity set at a cut-off of 0.25 WHO IU/mL); a diagnostic sensitivity (or correlation with a commercial reference kit manufactured in Europe and CE marked) of 100% was found.

### 16.3 Precision

It has been calculated on two samples, one negative and one low positive, examined in 16 replicates in three separate runs. CV values ranging between 5-10%, depending on OD<sub>450 nm</sub> values, were found. The variability seen did not result in sample misclassification.




## 17 LIMITATIONS

Frozen samples containing fibrin particles or aggregates after thawing have been observed to generate some false results.

**18 REFERENCES / LITERATURE**

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## SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
<b>IVD</b>	<i>In vitro</i> diagnostic medical device *	<i>In-vitro</i> -Diagnostikum *	Diagnostica in vitro	Diagnóstico in vitro	Diagnostic in vitro
<b>REF</b>	Catalogue number *	Artikelnummer *	No. di Cat.	No de catálogo	Référence
<b>LOT</b>	Batch code *	Chargencode *	Lotto no	Número de lote	No. de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservación	Température de conservation
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
<b>RUO</b>	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
<i>Distributed by</i>	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
<i>Content</i>	Content	Inhalt	Contenuto	Contenido	Conditionnement
<i>Volume/No.</i>	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité