HEV Ab
Version ULTRA
Elisa

Enzyme Immunoassay for the determination of total antibodies to Hepatitis E Virus in serum and plasma

Ref: KAPDEVABULTRA

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HEV Ab ULTRA

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the qualitative determination of total antibodies to Hepatitis E Virus (HEV) in serum and plasma. The kit is intended for the follow-up of HEV-infected patients and the screening of blood units.
In addition, due to the assay configuration of the product, the kit may be used also in testing total antibodies to HEV in serum and plasma derived from other not-human recipients for zoonotic studies.
For “in vitro” diagnostic use only.

B. 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.

C. PRINCIPLE OF THE TEST

Microplates are coated with highly specific synthetic antigen encoding for conservative and immunodominant determinants of HEV.
The solid phase is first treated with the sample where anti HEV total antibodies (mostly IgG, IgM and IgA) are captured, if present, by the antigens.
After washing out all the other components of the sample, in the second incubation bound anti HEV total antibodies are detected by the addition of the same HEV highly specific synthetic antigen labeled 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 antibodies present in the sample. After blocking the enzymatic reaction, its optical density is measured by an ELISA reader.
The version ULTRA is particularly suitable for automated screenings.

D. COMPONENTS

The standard configuration of the kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
n° 1 microplate. 12 strips of 8 breakable wells. Microplate is coated with HEV highly specific synthetic antigen. Plate is sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Negative Control CONTROL -
1x2.0ml/vial. Ready to use control. It contains bovine carrier proteins, 10 mM phosphate buffer pH 7.4 ± 0.1, 0.09% sodium azide and 0.045% ProClin 300. Yellowish colour coded.

3. Positive Control CONTROL +
1x2.0ml/vial. Ready to use control. It contains bovine carrier proteins, 10mM phosphate buffer pH 7.4 ± 0.1, inactivated human serum positive to HEV Ab and negative for HBsAg, HIV, Syphilis and HCV markers, 0.09% sodium azide and 0.045% ProClin 300. Green colour coded.

4. Calibrator: CAL …ml
N° 1 vials. Lyophilized calibrator. To be dissolved with the volume of EIA grade water reported on the label. It contains fetal bovine serum proteins, human antibodies to HEV whose content is calibrated on 1st 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 0.045% ProClin 300 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
1x60ml/bottle. 20x concentrated solution containing 0.045% ProClin 300 as preservative. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0 ± 0.2 and 0.05% Tween 20 .

6. Enzyme conjugate : CONJ
1x16 ml/bottle. Ready-to-use solution. It contains HEV specific synthetic antigen, labeled with HRP, 5% BSA, 10 mM Tris buffer pH 6.8 ± 0.1, 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives. Pink/red color coded

7. Chromogen/Substrate SUBS TMB
1x16ml/bottle.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 H2O2.
Note: To be stored protected from light as sensitive to strong illumination.

8. Sulphuric Acid: H2SO4 0.3 M
1x15ml/ bottle.Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

9. Plate sealing foil n° 2

10. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (200ul and 10ul) 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 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. 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..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. Do not reuse disposable tips.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
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 hrs or heat inactivation by autoclave at 121°C for 20 min..
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. 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.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venipuncture 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, clots 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°…+8°C in primary collection tubes for up to five days after collection.
   Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 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 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation.
In this case, call DIAsource's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Negative and Positive Controls:

Ready to use. Mix well on vortex before use.
Even if the HEV positive material used in the preparation of the positive control has been chemically inactivated and derived from HBsAg/HIVAb&Ag/HCVAb and Syph Ab negative material, handle such control as potentially infective.

Calibrator:

Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.

Important Notes:

- *The calibrator after dissolution is stable at +2..8°C for 1 month if properly handled. For longer storage store frozen in aliquots at –20°C and thaw only once.*
- *Even if the HEV positive material used in the preparation of the positive control has been chemically inactivated and derived from HBsAg/HIVAb&Ag/HCVAb and Syph Ab negative material, handle such control as potentially infective*

Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use.
During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic and possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred, use only plastic and possibly sterile disposable containers.

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).

Legenda:

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.

I. 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 ± 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°C (tolerance of ± 0.5°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 in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. **Incubation times** have a tolerance of ±5%.
5. The **ELISA reader** has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, mandatory) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0;(d) repeatability ≥ 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 workstation**, 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.
7. When using ELISA automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2..8°C, firmly capped.
8. DIAsource's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit, such the ELISA platform, **DIA.BLOod**, supplied by DiaPro already validated for the DiaPro's line of products.

L. 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 colorless 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 hr) 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 manufacturer's instructions. Set the right number of washing cycles as reported in the specific section.
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.

M. 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.

Automated assay:
In case the test is carried out automatically with an ELISA system, we suggest to dispense the sample 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 100 µl controls/calibrator in the appropriate control/calibration wells.

Important Note: *Visually monitor that samples have been dispensed into appropriate wells.*

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.
In case an automatic workstation is used, first assure that the instrument is validated according to point I.6.

Manual Assay:

1. Place the required number of microwells in the microwell holder. Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed.

2. Leave A1 well empty for the operation of blanking.
Dispense 100 µl of Negative Control in triplicate, and 100 µl of Positive Control in single in proper wells, followed by 100 µl of each of samples. In case the Calibrator is used (*) dispense 100 µl of it in a proper defined well in duplicate.
Do not dilute Controls and Calibrator as they are pre-diluted, ready to use!
Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm. (samples show ODvalues higher than 0.100).

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

3. Incubate the microplate for **45 min at 37°.**

4. Wash the microplate with an automatic washer as reported in section I.3.

5. Pipette 100 µl Enzyme Conjugate into each well, except the 1st blanking well, and cover with the sealer. Check that this pink/red coloured component has been dispensed in all the wells, except A1.

Important notes:
Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.

6. Incubate the microplate for **45 min at +37°C**.

7. Wash the microplate with an automatic washer as in step 4.

8. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature (18-24°C) for 15 minutes**.

Important note: *Do not expose to strong direct light as a high background might be generated.*

9. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 8 to stop the enzymatic reaction. Addition of acid will turn the positive control and positive samples from blue to yellow/brown.

10. Measure the color intensity of the solution in each well, as described in section I.5, with a microplate reader at 450nm (reading) and at 620-630nm (background Subtraction), blanking the instrument on A1 well.

Important notes:
1. *Ensure that no finger prints are present on the bottom of the microwell before reading. 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.*
3. *Shaking at 350 ± 150 rpm during incubation has been proved to increase the sensitivity of the assay of about 20%.*

N. ASSAY SCHEME

Method	Operations
Controls & Calibrator (*) Samples	100 µl 100 µl
1st incubation	**45 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 µl
2nd incubation	**45 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Chromogen/Substrate	100 µl
3rd incubation	**15 min**
Temperature	r.t.
Sulphuric Acid	100 µl
Reading OD	450nm/620-630nm

(*) 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) might be used only whenever 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	S4										
B	NC	S5										
C	NC	S6										
D	NC	S7										
E	PC	S8										
F	S1	S9										
G	S2	S10										
H	S3	S11										

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

O. INTERNAL QUALITY CONTROL

A check is carried out on the controls any time the kit is used in order to verify whether their OD450nm/620-630nm values are as expected and reported in the table below.

Check	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.150 mean OD450nm value after blanking
Positive Control (PC)	> 1.000 OD450nm 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
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control (NC) > 0.150 OD450nm 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.
Positive Control < 1.000 OD450nm	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 OD450nm 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.

Important Note:

If Calibrator has been used, verify the following data:

Check	Requirements
Calibrator	S/Co > 1.5

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

Problem	Check
Calibrator S/Co < 1.5	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (e.g..: 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.

Important note:

The analysis must be done proceeding as the reading step described in the section M, point 10.

P. CALCULATION OF THE CUT-OFF

The tests results are calculated by means of a cut-off value determined with the following formula on the mean OD450nm/620-630nm value of the Negative Control (NC):

NC + 0.250 = Cut-Off (Co)

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.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as ratio of the sample OD450nm/620-630nm 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. *Any positive result should be confirmed by an alternative method capable to detect anti HEV antibodies with a different technology.*

3. *When comparing the results of the assay with any other commercial CE/FDA marked product be reminded that the DIAsource kit detects all the antibodies including IgM and IgA and not only IgG.*
4. *When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.*
5. *Diagnosis of HEV infection has to be done and released to the patient only by a qualified medical doctor.*
6. *In case the kit is used with samples of animal origin it will be responsibility of the Lab Manager to assign a proper cut-off to the system and then calculate results.*

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 10):

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

Negative Control: 0.049 – 0.050 – 0.051 OD450nm
Mean Value: 0.050 OD450nm
Lower than 0.150 – Accepted

Positive Control: 2.589-2.591 OD450nm
Mean Value: 2.590 OD450nm
Higher than 1.000 – Accepted

Cut-Off = 0.050+0.250 = 0.300
Calibrator: 0.930 - 0.936 OD450nm
Mean value: 0.933 OD450nm S/Co = 3.1
S/Co higher than 1.5 – Accepted

Sample 1: 0.070 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

R. PERFORMANCES

1. LIMIT OF DETECTION

It was assessed on the 1st WHO reference reagent for HEV antibody, NIBSC code 95/584. The preparation was diluted in a pooled human HEV Ab negative serum and then tested in three lots. Results are reported in the table below:

IU/ml	P 1 $_{mean}$OD450	P 2 $_{mean}$OD450	P 3 $_{mean}$OD450	Average OD450	Average STD
2.0	2,442	2,456	2,452	2,450	0,088
1.0	1,305	1,301	1,297	1,301	0,111
0.5	0,587	0,600	0,603	0,597	0,019
0.25	0,269	0,273	0,272	0,272	0,029
0.125	0,131	0,129	0,127	0,129	0,007
0.062	0,068	0,070	0,065	0,068	0,008
0.031	0,036	0,034	0,034	0,035	0,005
0	0,009	0,008	0,004	0,007	0,006

The limit of detection, calculated as Negative Control (0 IU/ml) mean OD450nm + 5xSTD, turned out to be far and far better than **0.25 WHO IU/ml.**

2. DIAGNOSTIC PERFORMANCES

The studies were carried out considering a cut-off set at 0.25 WHO IU/ml**.**

2.1 Diagnostic Sensitivity:

It was calculated on a panel of positive samples prescreened with a CE marked commercial product for the determination of anti HEV Ab (but prevalently IgG as reported in their IFU) and on two commercially available sero-conversions.

Results are reported in the table below for these two panels (Biomex – Germany).

	HEV Ab version ULTRA		REFERENCE KIT	
	$_{mean}$OD450	S/CO	$_{mean}$OD450	S/CO
SCP-HEV-006b No 1	0,015	0,1	0,000	0,0
SCP-HEV-006b No 2	0,010	0,0	0,000	0,0
SCP-HEV-006b No 3	0,011	0,0	0,000	0,0
SCP-HEV-006b No 4	0,016	0,1	0,000	0,0
SCP-HEV-006b No 5	0,015	0,1	0,001	0,0
SCP-HEV-006b No 6	0,009	0,0	0,000	0,0
SCP-HEV-006b No 7	0,010	0,0	0,000	0,0
SCP-HEV-006b No 8	0,592	**2,3**	0,013	0,1
SCP-HEV-006b No 9	2,859	**11,1**	1,868	**9,8**
SCP-HEV-006b No 10	1,755	**6,8**	1,972	**10,4**
SCP-HEV-006b No 11	3,526	**13,7**	2,866	**15,1**
SCP-HEV-006b No 12	3,625	**14,1**	2,459	**12,9**
SCP-HEV-006b No 13	3,712	**14,4**	2,959	**15,6**
SCP-HEV-006b No 14	3,703	**14,4**	2,857	**15,0**
SCP-HEV-006b No 15	3,609	**14,0**	3,130	**16,5**
SCP-HEV-006b No 16	3,749	**14,5**	3,068	**16,1**
SCP-HEV-006b No 17	3,700	**14,3**	3,160	**16,6**
SCP-HEV-006b No 18	3,650	**14,1**	3,096	**16,3**
SCP-HEV-006b No 19	3,695	**14,3**	2,776	**14,6**
SCP-HEV-006b No 20	over	**> 19**	3,032	**16,0**
SCP-HEV-006b No 21	over	**> 19**	3,170	**16,7**
SCP-HEV-006b No 22	3,852	**14,9**	3,224	**17,0**
SCP-HEV-006b No 23	over	**> 19**	3,280	**17,3**

	HEV Ab version ULTRA		REFERENCE KIT	
	$_{mean}$OD450	S/CO	$_{mean}$OD450	S/CO
SCP-HEV-001b No 1	0,008	0,0	0,000	0,0
SCP-HEV-001b No 2	0,006	0,0	0,000	0,0
SCP-HEV-001b No 3	1,049	**4,1**	0,007	0,0
SCP-HEV-001b No 4	3,487	**13,5**	0,918	**4,8**
SCP-HEV-001b No 5	3,236	**12,5**	2,160	**11,4**
SCP-HEV-001b No 6	2,377	**9,2**	3,074	**16,2**
SCP-HEV-001b No 7	2,113	**8,2**	3,310	**17,4**
SCP-HEV-001b No 8	3,577	**13,9**	3,875	**20,4**

Samples SCP-HEV-006b No 8 and SCP-HEV-001b No 3 turned out to be positive for anti HEV IgM with the product code KAPDEVM.
The overall value found for the Diagnostic Sensitivity was 100%.

2.2 Diagnostic Specificity

It was calculated on a panel of negative samples prescreened with a CE marked commercial product for the determination of anti HEV Ab (but prevalently IgG as reported in their IFU) and on a panel of potential interferents.
No interference was seen in such samples and the overall value found for the Diagnostic Specificity was > 99.5.
The resulting 0.5% false positive samples turned out to be mostly IgM and/or IgA positive.

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 OD450nm values, were found. The variability seen did not result in sample misclassification.

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 10.

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

REFERENCES
1. Tian-Cheng Li et al., Journal of Virology, 1997, p 7207-7213
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4. M.Fogeda et al., 2012, 84, p 71-74
5. Saskia A. et al., EID Journal, 2009, 15/3,

All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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