HBe Ag&Ab

Enzyme Immunoassay (ELISA) for the determination of Hepatitis B Virus "e" Antigen and Antibody in human plasma and sera.

- for “in vitro” diagnostic use only -

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REF. HBE.CE
96 Tests

HBe Ag&Ab

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of Hepatitis B Virus "e" Antigen and Antibody in human plasma and sera.

The kit is intended for the follow-up of acute infection and of chronic patients under therapy.

For “in vitro” diagnostic use only.

B. INTRODUCTION

Hepatitis B “e” Antigen or HBeAg is known to be intimately associated with Hepatitis B Virus or HBV replication and the presence of infectious Dane particles in the blood.

Recently, it has been found that HBeAg is a product of proteolytic degradation of Hepatitis B core Antigen or HBcAg, occurring in hepatocites, whose expression is under the control of the precore region of HBV genome.

If HBeAg is considered a specific marker of infectivity, the presence of anti HBeAg antibodies in blood is recognised to be a clinical sign of recovery from infection to convalescence.

The determination of these two analytes in samples from HBV patients has become important for the classification of the phase of illness and as a prognostic value in the follow up of infected patients.

C. PRINCIPLE OF THE TEST

HBeAg:

HBeAg, if present in the sample, is captured by a specific monoclonal antibody, in the 1st incubation.

In the 2nd incubation, after washing, a tracer, composed of a mix of two specific anti HBeAg monoclonal antibodies, labeled with peroxidase (HRP), is added to the microplate and binds to the captured HBeAg.

The concentration of the bound enzyme on the solid phase is proportional to the amount of HBeAg in the sample and its activity is detected by adding the chromogen/substrate in the 3rd incubation.

The presence of HBeAg in the sample is determined by means of a cut-off value that allows for the semiquantitative detection of the antigen.

HBeAb

Anti HBeAg antibodies, if present in the sample, compete with a recombinant HBeAg preparation for a fixed amount of an anti HBeAg antibody, coated on the microplate wells.

The competitive assay is carried out in two incubations, the first with the sample and recHBeAg, and the second with a tracer, composed of two anti HBeAg monoclonal antibodies, labeled with peroxidase (HRP).

The concentration of the bound enzyme on the solid phase becomes inversely proportional to the amount of anti HBeAg antibodies in the sample and its activity is detected by adding the chromogen/substrate in the third incubation.

The concentration of HBeAg specific antibodies in the sample is determined by means of a cut-off value that allows for the semi quantitative detection of anti HBeAg antibodies.

D. COMPONENTS

The kit contains reagents for total 96 tests.

1. Microplate: MICROPLATE

n° 1 coated microplate

12 strips of 8 breakable wells coated with anti HBeAg specific monoclonal antibody, postcoated with bovine serum proteins and 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 2..8°C.

2. Negative Control: CONTROL -

1x2.0ml/vial. Ready to use control. It contains bovine serum, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The negative control is colorless.

3. Antigen Positive Control: CONTROL + Ag

1x1.0ml/vial. Ready to use control. It contains 2% bovine serum albumin, non infectious recombinant HBeAg, 100 mM tris buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.

The positive control is green color coded.

4. Antibody Positive Control: **:** CONTROL + Ab

1x1.0ml/vial. Ready to use control. It contains 2% bovine serum albumin, human anti HbeAg positive plasma at about 10 PEI U/ml, 100 mM tris buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The label is red colored.

The positive control is yellow color coded.

5. Antigen Calibrator: CALAG …ml

n° 1 vial. Lyophilised calibrator for HBeAg. To be dissolved with EIA grade water as reported in the label. It contains fetal bovine serum, non infectious recombinant HBeAg at 1 PEI U/ml +/- 10%, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives.

Important 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.

6. Antibody Calibrator: CALAB …ml

n° 1 vial. Lyophilized calibrator for anti HBeAg antibody. To be dissolved with EIA grade water as reported in the label. It contains fetal bovine serum, positive plasma at 0.25 PEI U/ml +/-10%, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives. The label is red colored.

Important 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.

7. Wash buffer concentrate: WASHBUF 20X

1x60ml/bottle. 20x concentrated solution.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

8. Enzyme conjugate: CONJ

1x16ml/vial. Ready to use conjugate. It contains Horseradish peroxidase conjugated with a mix of monoclonal antibodies to HBeAg, 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

The reagent is red color coded.

9. HBe Antigen: Ag-HBe

1x10ml/vial. Ready to use reagent. It contains recombinant HBeAg, fetal bovine serum, buffered solution pH 8.0+/-0.1, 0.045% ProClin 300 and 0.09% sodium azide as preservatives.

The reagent is blue color coded.

10. Chromogen/Substrate: SUBS TMB

1x16ml/vial. Ready-to-use component. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H_2O_2.

Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H_2SO_4 O.3 M

1x15ml/vial. It contains 0.3 M H2SO4 solution.

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

12. Plate sealing foils n°2

13. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled 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 (dry or wet) set at +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. 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.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. 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 (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.
6. 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.
7. 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.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
11. 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.
12. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
13. 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. 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..
14. Accidental spills 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.
15. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water
16. 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 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; 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.
4. Haemolysed 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°…+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

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

1. Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro'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.
When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Antigen Positive Control:
Ready to use. Mix well on vortex before use.

4. Antibody Positive Control:
Ready to use. Mix well on vortex before use.

5. Antigen Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.

Note: The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.

6. Antibody Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.
Note: The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.

7. 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.
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.

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

9. HBe Antigen:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

10. 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 if possible, sterile disposable container.

11. 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. Decontamination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
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 $\pm$5%.
5. The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. 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 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 section "Internal Quality Control''. 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 when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro'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.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+H_2O_2) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation

and no spillage of liquid is present inside the box (primary container). Check that the aluminium 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 and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
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 reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn 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.

In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be performed according to the procedure given below, taking care to maintain the same incubation time for all the samples being tested.

A) HBe Antigen:

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Pipette 100 µl of the Negative Control in triplicate, 100 µl of the Antigen Calibrator in duplicate and then 100 µl of the Antigen Positive Control in single.
4. Then dispense 100 µl of samples in the proper wells.
5. Check for the presence of samples in wells by naked eye (there is a marked colour difference between empty and full wells) or by reading at 450/620nm (samples show OD values higher than 0.100).
6. Incubate the microplate for **60 min at +37°C**.

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.*

7. When the first incubation is finished, wash the microwells as previously described (section I.3)
8. Dispense 100 µl Enzyme Conjugate in all wells, except for A1, used for blanking operations.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.*

9. Check that the reagent has been dispensed properly and then incubate the microplate for **60 min at +37°C**.
10. When the second incubation is finished, wash the microwells as previously described (section I.3)
11. Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

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

12. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive control and positive samples will turn from clear to blue.
13. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 11. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
14. Measure the color intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

B) HBe Antibody:

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Pipette 50 µl of the Negative Control in triplicate, 50 µl of the Antibody Calibrator in duplicate and then 50 µl of the Antibody Positive Control in single.
4. Then dispense 50 µl of samples in the proper wells.
5. 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 OD values higher than 0.100).
6. Dispense then 50 µl of HBe Antigen in all the wells, except for A1.
7. Incubate the microplate for **60 min at +37°C**.

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.*

8. When the first incubation is finished, wash the microwells as previously described (section I.3)
9. Finally proceed as described for the HBeAg assay from point 8 to the last one.

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 should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

HBe antigen test

Controls and calibrator Samples	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

HBe antibody test

Controls and calibrator Samples	50 ul 50 ul
Neutralising antigen	50 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzymatic conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mixture	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

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
PC = Positive Control // CAL = Calibrators // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

HBe Antigen

Check	OD450nm
Blank well	< 0.100 OD450nm
Negative Control (NC)	< 0.150 OD450nm after blanking coefficient of variation < 30%
Antigen Calibrator	S/Co > 2.0
Positive Control (PC)	> 1.500 OD450nm

HBe Antibody

Check	OD450nm
Blank well	< 0.100 OD450nm
Negative Control (NC)	> 1.000 OD450nm after blanking coefficient of variation < 10%
Antibody Calibrator	OD450nm < NC/1.5
Positive Control (PC)	OD450nm < NC/10

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

If they do not, don't proceed any further and perform the following checks:

HBeAg

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.150 OD450nm after blanking coefficient of variation > 30%	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 the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator S/Co < 2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead); 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.
Positive Control < 1.500 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control); 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.

HBe antibody

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD450nm after blanking coefficient of variation > 10%	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 (e.g.: dispensation of positive control instead of negative control; no dispensation of the Neutralizing Antigen; no dispensation of the Enzyme Conjugate); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred; 5. that micropipettes have not become contaminated with positive samples; 6. that the washer needles are not blocked or partially obstructed.

Calibrator OD450nm > NC/1.5	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead; no dispensation of the Neutralizing Antigen; no dispensation of the Enzyme Conjugate); 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.
Positive Control OD450nm > NC/10	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control; 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.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 14.

P. CALCULATION OF THE CUT-OFF
The results are calculated by means of a cut-off value determined with the following formula:

HBeAg:

NC + 0.100 = Cut-Off (Co)

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

HBeAb:

(NC + PC) / 3 = Cut-Off (Co)

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS
Results are interpreted as follows:

HBeAg:

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

HBeAb:

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

Note:
S = OD450nm/620-630nm of the sample
Co = cut-off value

An example of calculation for HBeAg assay is reported below (data obtained proceeding as the the reading step described in the section M, point 14):

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

Negative Control: 0.020 – 0.030 – 0.025 OD450nm
Mean Value: 0.025 OD450nm
Lower than 0.150 – Accepted

Positive Control: 2.489 OD450nm
Higher than 1.500 – Accepted
Cut-Off = 0.025+0.100 = 0.125
Calibrator: 0.520 - 0.540 OD450nm
Mean value: 0.530 OD450nm S/Co = 4.2
S/Co higher than 2.0 – Accepted

Sample 1: 0.030 OD450nm
Sample 2: 1.800 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

An example of calculation for HBeAb is reported below (data obtained proceeding as the the reading step described in the section M, point 14):

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

Negative Control: 2.100 – 2.200 – 2.000 OD450nm
Mean Value: 2.100 OD450nm
Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm
Lower than NC/10 – Accepted

Cut-Off = (2.100 + 0.100) / 3 = 0.733
Calibrator: 0.720-0.760 OD450nm
Mean value: 0.740 OD450nm
OD450nm < NC/1.5 – Accepted

Sample 1: 0.020 OD450nm
Sample 2: 1.900 OD450nm
Sample 1 Co/S > 1.1 positive
Sample 2 Co/S < 0.9 negative

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory director to reduce the risk of judgment errors and misinterpretations.*
2. *The Identification of the clinical status of a HBV patient (acute, chronic, asymptomatic hepatitis) has to be done on the basis also of the other markers of HBV infection (HBsAg, HBsAb, HBcAb, HBcIgM);*
3. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

A) HBeAg

1. Limit of detection
The limit of detection of the assay has been calculated by means of the International Standard for HBeAg, supplied by Paul Erlich Institute (PEI).
The data obtained by examining the limit of detection on three lots is reported in the table below.

HBE.CE Lot ID	PEI U/ml HBeAg
0103	0.25
0103/2	0.25
0303	0.25

In addition the preparation Accurun # 51, produced by Boston Biomedica Inc., USA, has been tested, upon dilution in FCS. Results are reported for three lots of products.

BBI's Accurun 51 (S/Co)

HBE.CE Lot ID	1 x	2 x	4 x	8 x	16x
0103	**4.1**	**1.6**	0.9	0.6	0.4
0103/2	**4.1**	**1.7**	0.9	0.6	0.4
0303	**4.0**	**1.6**	0.9	0.5	0.4

2. Diagnostic Sensitivity:
The diagnostic sensitivity has been tested on panels of samples classified positive by a US FDA approved kit.
Positive samples were collected from different HBV pathologies (acute, chronic) bearing HBeAg reactivity.
An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.
Moreover the Panel of Seroconversion code PHM 935B, produced by BBI, was examined.
Data are reported below and compared with those reported by BBI for two other commercial products.

Sample ID	HBE.CE S/Co	Abbott EIA S/Co	Sorin EIA S/Co
21	5.4	4.5	6.3
22	3.7	4.3	5.4
23	1.9	3.2	3.1
24	1.1	2.4	1.5
25	1.0	2.1	1.2
26	0.6	1.7	0.7
27	0.2	0.8	0.3
28	0.2	0.6	0.2
29	0.2	0.4	0.2
30	0.2	0.3	0.2
31	0.1	0.3	0.2
32	0.1	0.3	0.2

Finally the Performance Panel code PHJ 201, produced by BBI, was tested. Data are reported below and compared with those reported by BBI for an other commercial product.

Member	PEI U/ml	HBE.CE	Sorin EIA
1	3	3.3	7.0
2	6	17.5	21.9
3	26	30.1	37.1
4	31	29.4	23.5
5	1	1.1	2.2
6	2	2.3	6.9
7	35	30.1	24.6
8	38	29.2	31.9
9	4	16.6	10.8
10	-	0.3	0.2
11	1	3.4	3.6
12	< 1	0.2	1.2
13	< 1	0.9	1.4
14	-	0.2	0.2
15	-	0.4	0.1
16	-	0.5	0.1
17	-	0.3	0.2
18	-	0.2	0.2
19	-	0.2	0.1
20	-	0.2	0.1
21	-	0.3	1.0
22	-	0.3	0.1
23	-	0.4	0.1
24	-	0.2	0.2
25	-	0.3	0.2

3. Diagnostic Specificity:
The diagnostic specificity has been determined on panels of negative samples from normal individuals and blood donors, classified negative with a FDA approved kit.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.
Samples derived from patients with different viral (HCV and HAV) and non viral pathologies of the liver that may interfere with the test were examined.
No cross reaction were observed.
The Performance Evaluation study conducted in a qualified external reference center on more than 500 samples has provided a value > 98% .

4. Precision
It has been calculated on two samples examined in 16 replicate in three different runs on three lots.
The values found were as follows:

HBE.CE: lot # 0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.030	0.027	0.032	0.029
Std.Deviation	0.002	0.002	0.003	0.002
CV %	7.4	8.2	7.9	7.8

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.569	0.559	0.575	0.568
Std.Deviation	0.027	0.029	0.028	0.028
CV %	4.7	5.3	4.9	4.9
S/Co	4.4	4.4	4.4	4.4

HBE.CE: lot # 0103/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.033	0.031	0.030	0.032
Std.Deviation	0.003	0.003	0.002	0.003
CV %	7.9	8.5	7.4	8.0

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.565	0.573	0.568	0.569
Std.Deviation	0.026	0.025	0.024	0.025
CV %	4.7	4.3	4.2	4.4
S/Co	4.2	4.4	4.4	4.3

HBE.CE: lot # 0303

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.029	0.034	0.038	0.034
Std.Deviation	0.003	0.003	0.004	0.003
CV %	9.7	9.8	9.2	9.6

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.579	0.573	0.564	0.572
Std.Deviation	0.023	0.028	0.025	0.025
CV %	4.1	4.8	4.5	4.5
S/Co	4.5	4.3	4.1	4.3

B) HBe Antibody

1. Limit of detection

The limit of detection of the assay has been calculated by means of the International Standard for HBeAb, supplied by Paul Erlich Institute (PEI).

The data obtained by examining the limit of detection on three lots is reported in the table below.

HBE.CE Lot ID	PEI U/ml HBeAb
0103	0.25
0103/2	0.25
0303	0.25

In addition the preparation Accurun # 52, produced by Boston Biomedica Inc., USA, has been tested, upon dilution in FCS. Results are reported for three lots of products.

Accurun 52 (Co/S)

HBE.CE Lot ID	1 x	2 x	4 x	8 x	16x
0103	1.0	0.8	0.6	0.4	0.4
0103/2	1.0	0.8	0.6	0.5	0.4
0303	1.0	0.8	0.6	0.4	0.4

2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested on panels of samples classified positive for HBeAb by a US FDA approved kit.

Positive samples were collected from different HBV pathologies bearing anti HBeAg antibody reactivity.

An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.

Moreover the Panel of Seroconversion code PHM 935B, produced by BBI, was examined.

Data are reported below and compared with those reported by BBI for two other commercial products.

Sample ID	HBE.CE Co/S	Abbott EIA Co/S	Sorin EIA Co/S
21	0.4	0.4	0.5
22	0.4	0.5	0.6
23	0.4	0.6	0.5
24	0.4	0.5	0.6
25	0.4	0.6	0.5
26	0.5	0.6	0.6
27	0.6	0.8	0.7
28	0.7	0.9	0.7
29	0.6	0.9	0.7
30	0.8	1.0	0.9
31	1.0	1.3	1.1
32	1.0	1.2	1.0

Finally the Performance Panel code PHJ 201, produced by BBI, was tested. Data are reported below and compared with those reported by BBI for another commercial product.

Member	PEI U/ml	HBE.CE	Sorin EIA
1	-	0.3	0.5
2	-	0.2	0.5
3	-	0.2	0.5
4	-	0.2	0.5
5	-	0.3	0.6
6	-	0.3	0.6
7	-	0.2	0.4
8	-	0.2	0.4
9	-	0.2	0.5
10	-	1.9	0.6
11	-	0.3	0.5
12	-	0.4	0.9
13	2	4.4	9.1
14	1	3.8	2.9
15	< 1	1.0	1.5
16	> 50	4.3	120.9
17	< 1	1.0	1.0
18	5	5.6	21.8
19	1	2.7	6.4
20	11	5.0	47.3
21	2	1.9	10.0
22	26	28.1	90.7
23	-	0.3	0.5
24	< 1	0.8	1.3
25	50	28.1	167.4

3. Diagnostic specificity:

The clinical specificity has been determined as described before for HBeAg.

The Performance Evaluation study conducted in a qualified external reference center on more than 500 samples has provided a value > 98% .

4. Precision:

It has been calculated on two samples examined in 16 replicate in three different runs on three lots.

The values found were as follows:

HBE.CE: lot # 0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.484	2.420	2.471	2.458
Std.Deviation	0.129	0.160	0.142	0.144
CV %	5.2	6.6	5.7	5.9

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.867	0.800	0.878	0.848
Std.Deviation	0.043	0.060	0.050	0.051
CV %	5.0	7.5	5.7	6.1
Co/S	1.0	1.0	1.0	1.0

HBE.CE: lot # 0103/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.316	2.361	2.413	2.363
Std.Deviation	0.127	0.144	0.146	0.139
CV %	5.5	6.1	6.0	5.9

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.767	0.793	0.785	0.781
Std.Deviation	0.041	0.050	0.046	0.046
CV %	5.4	6.3	5.8	5.8
Co/S	1.0	1.0	1.0	1.0

HBE.CE: lot #0303

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.334	2.415	2.437	2.395
Std.Deviation	0.146	0.155	0.158	0.153
CV %	6.3	6.4	6.5	6.4

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.850	0.867	0.876	0.864
Std.Deviation	0.052	0.051	0.048	0.050
CV %	6.1	5.9	5.5	5.8
Co/S	0.9	1.0	1.0	1.0

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 14.

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. 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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