HBe Ag & Ab
Enzyme Immunoassay Test
Product No. 4230

SUMMARY OF PROCEDURE

1. STEP 1 (Antigen): Place 100 µl of Controls, Calibrator and samples in the wells of the strips, leaving one well for the blank.
   STEP 1 (Antibody): Place 50 µl of Controls, Calibrator and samples in the wells of the strips, leaving one well for the blank. Then dispense 50 µl of HBe Antigen in all wells, except in the blank.
2. Incubate for 60 min. at 37°C
3. Wash 4-5 times (350 µl)
4. STEP 2 Place 100 µl of Enzyme Conjugate in each well
5. Incubate for 60 min. at 37°C
6. Wash 4-5 times (350 µl)
7. STEP 3 Place 100 µl of Chromogen/Substrate in each well
8. Incubate for 20 min. at room temperature (18-30°C)
9. STEP 4 Add 100 µl of Stop Solution
10. Read absorbance at 450 nm & 620 nm.

INTENDED USE

Enzyme immunoassay for the detection of Hepatitis B Virus "e" Antigen and Antibody (HBeAg, HBeAb) in human serum or plasma. The kit is intended for the follow-up of acute infection and of chronic patients under therapy.
For "*in vitro*" diagnostic use only.

SUMMARY AND EXPLANATION

The Hepatitis B Virus (HBV) is a human pathogen DNA virus with a word-wide distribution among geographic areas and population groups. He is one of the five strictly epatotropic viruses, together with the A, C, Delta and E viruses. All of these viruses can cause acute disease, with symphtoms that ranged between mild asymphtomatic infection to severe fulminant hepatitis[1-3]. The thpical symptoms are yellowing of the skin and eyes (jaundice), dark urine and extreme fatigue, nausea, vomiting and abdominal pain[4]. HBV can cause chronic infection when the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer[4], particularly if the infection was acquired in childhood than as an adult[5,6]. In this case the infection persists for more than six months. More than 90% of the infected adults will have an acute self-limiting infection[1]. Young children are the most likely to develop chronic hepatitis infection. 90% of the infants affected by HBV infection in the first year and 30-50% of the infants affected into the first 4 years can develop chronic hepatitis, and 25% of these patients will death for liver cancer or cirrhosis[4]. HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Since 1991, WHO suggested to consider the hepatitis B vaccine into their national immunization programs. The therapy is mainly based on alfa-interferon to relieve symptoms[5,6]. The response to this treatment is between 40 and 50% of patients with chronic active HBV infection[5,6]. In the chronic HBV carriers there is no evidence of hepatic damage[1,2], the infection persists and the patient maintains the ability to transmit the virus.
The transmission of the disease is mainly due to a parenteral contac (blood or blood products exchange, sexual contacts, perinatal spread from mother to newborn at the birth, unsafe injections and transfusions)[4]. The virus is not spread by contaminated food or water. High prevalence of HBV infections are found in Southern areas of Eastern and Central Europe. Lower prevalence (5%) is detected in Middle and Far East, and less that 1% in Western Europe and North America[4].
In the HBV infection three phases can be identify: incubation, acute and covalescent, on the basis of several serological markers results. The first marker to appear in the serum is the hepatitis B surface antigen (HBsAg),m detectable after 4-12 weeks after the infection and before the onset of the symptoms[1,2,5].
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

PRINCIPLE OF THE PROCEDURE

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 anti-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.

REAGENTS

Reagents are sufficient for 96 determinations
Bring to room temperature before use

Antibody Coated Wells (Microplate)	12 x 8 breakable wells coated with monoclonal antibodies specific to HBeAg postcoated with bovine serum proteins and sealed into a bag with desiccant.
Negative Control	One vial containing 1.8 ml of ready to use 2% BSA into 100 mM Tris-HCl buffer pH 7.4+/-0.1 and 0.1% Kathon GC as preservative. The negative control is colourless.
Antigen Positive Control	One vial containing 1.0 ml of ready to use non infectious recombinant HBeAg, with 2% BSA, 100 mM Tris buffer pH 7.4±0.1, and 0.1% Kathon GC as preservative. The Antigen Positive Control is green colour coded.

Antibody Positive Control — One vial containing 1.0 ml of ready to use anti-HBe positive plasma at about 10 PEI U/ml, with 2% BSA, 100 mM Tris buffer pH 7.4±0.1 and 0.1% Kathon GC as preservative.
The Antibody Positive Control is yellow colour coded.
Important Note: Even if plasma has been chemically inactivated, handle this component as potentially infectious.

Antigen Calibrator — One vial of lyophilized reagent for HBeAg. To be reconstitute with EIA grade water as reported in the label. Contains 4% bovine serum proteins, non infectious recombinant HBeAg at 1 PEI U/ml ± 10%, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.
Note: The volume necessary to reconstitute the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

Antibody Calibrator — One vial of lyophilized reagent for HBeAb. To be reconstitute with EIA grade water as reported in the label. Contains 4% bovine serum proteins, positive plasma at 0.25 PEI U/ml ± 10%, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.
Note: The volume necessary to reconstitute the content of the vial may vary from lot to lot. Please use the right volume reported on the label.
Important Note: Even if plasma has been chemically inactivated, handle this component as potentially infectious.

Wash buffer 20X concentrate — One vial containing 60 ml of 20X concentrated solution. Once diluted, the working solution contains 10 mM phosphate buffer pH 7.0 ± 0.2, 0.05% Tween 20 and 0.1% Kathon GC.

Enzyme Conjugate — One vial containing 16 ml of ready to use Horseradish Peroxidase (HRP) conjugated with a mix of monoclonal antibodies to HBeAg, MOPS buffer pH 6.2 - 6.7.Contains bovine protein preserved with 0.02% methylisothiazolone and bromonitrodioxane, 20 ppm Proclin 300 .
The reagent is red color coded.

HBe Antigen — One vial containing 10 ml of ready to use recombinant HBeAg, fetal bovine serum, buffered solution pH 8.0±0.1, 0.1% Kathon GC and 0.09% sodium azide as preservatives.
The reagent is blue color coded.

Chromogen Substrate — One vial contains **20** ml of ready to use 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethyl-sulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

Stop Solution — One vial contains **20** ml ready to use of 0.3 M H_2SO_4 solution.

Adhesive sealing foils

OTHER MATERIALS REQUIRED

Calibrated Micropipettes (150 µl, 100 µl and 50 µl) and disposable plastic tips.
EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
Timer with 60 minute range or higher.
Absorbent paper tissues.
Calibrated ELISA microplate thermostatic incubator (dry or wet), capable to provide shaking at 1300 rpm+/-150, set at +37°C.
Calibrated ELISA microwell reader with 450 nm (reading) and possibly with 620-630 nm (blanking) filters.
Calibrated ELISA microplate washer.
Vortex or similar mixing tools.

STORAGE AND STABILITY OF REAGENTS

Reagents must be stored at 2/8°C.
The expiry date is printed on each component and on the box label.
Reagents have a limited stability after opening and/or preparation

Microplate Use: open the package on the opposite end from the code which is useful for identification purposes, remove the support and strips to be used from the foil package, and place at 2-8°C the unused strips in the polythene bag with the dessiccant, expell the air and seal by pressing the closure.

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

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

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

Antigen Calibrator Add the volume of EIA grade water reported on the label to the lyophilized powder. Let fully dissolve and gently mix on vortex.
Note: The solution is not stable. Store the Antigen Calibrator frozen in aliquots at -20°C.

Antibody Calibrator Add the volume of EIA grade water reported on the label to the lyophilized powder. Let fully dissolve and gently mix on vortex.
Note: The solution is not stable. Store the Antigen Calibrator frozen in aliquots at -20°C.

Wash buffer concentrate The whole content of the concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution. 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 +2-8° 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 if possible, sterile disposable containers.

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.

Chromogen Substrate Ready to use. Mix well on vortex before use. Avoid contamination of the liquid with oxidizing chemicals, airdriven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces. If this components has to be transferred use only platic and, if possible, sterile disposable container.

Stop Solution Ready to use. Mix well on vortex before use.

WARNINGS AND PRECAUTIONS

For in vitro Diagnostic Use Only.
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 (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 for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. 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 **3** months.
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 liquid components or in transferring components into automated workstations, in order to avoid cross 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 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..
14. 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.
15. 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.
16. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
17. The ELISA incubator has to be set at +37°C (tolerance of $\pm$1°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.
18. 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/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350 µl/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/calibrator and well-characterized negative and positive reference samples, and check to match the values reported below in the section "QUALITY CONTROL". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
19. Incubation times have a tolerance of ±5%.
20. The microplate reader has to be equipped with a reading filter of 450 nm and ideally with a second filter (620-630 nm) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 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.
21. When using ELISA automated workstations, all critical steps (dispensation, incubation, washing, reading, shaking, data handling, etc.) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "QUALITY CONTROL". The assay protocol has to be installed in the operating system of the unit and validated by checking full matching the declared performances of the kit. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set paying particular attention to avoid carry over by the needles used for dispensing samples and for washing. The carry over effect must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.

SPECIMEN COLLECTION

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 lipemic ("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 well as they could give rise to false positive results.
5. Sera and plasma can be stored at +2°-8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen sample 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 µ filters to clean up the samples for testing.

PROCEDURE

PREASSAY PROCEDURE

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. Reconstitute the Antigen & Antibody 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 man-

ufacturers 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.

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.

HBe Antigen detection

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 450/620 nm (samples show OD values higher than 0.100).
6. Incubate the microplate for 60 min at +37°C.
7. When the first incubation is finished, wash the microwells as previously described (section "WARNINGS AND PRECAUTIONS")
8. Dispense 100 µl Enzyme Conjugate in all wells, except for A1, used for blanking operations.
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 "WARNINGS AND PRECAUTIONS")
11. Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.
12. Incubate the microplate protected from light at **room temperature** for 20 minutes. Wells dispensed with positive control and positive samples will turn from clear to blue.
13. Pipette 100 µl Stop Solution 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 colour intensity of the solution in each well, as described in section "WARNINGS AND PRECAUTIONS", using a 450 nm filter (reading) and if possible a 620-630 nm filter (background subtraction), blanking the instrument on A1.

HBe Antibody detection

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 450/620 nm (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.
8. When the first incubation is finished, wash the microwells as previously described (section "WARNINGS AND PRECAUTIONS")
9. Finally proceed as described for the HBeAg assay from point 8 to the last one.

IMPORTANT NOTES

1. *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.*
2. *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.*
3. *Do not expose to strong direct light as a high background might be generated.*
4. *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.*
5. *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.*

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 = Calibrator; S = Samples

QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the expected OD_{450nm} or S/Co values have been matched in the analysis.
Control that the following data are matched:

HBe Antigen

Blank well (Bw): OD_{BW} < 0.100;
Negative Control (NC): OD_{NC} value after blanking < 0.150; CV% < 30%
Antigen Calibrator: S/CO > 2;
Positive Control (PC): OD_{PC} > 1.500

HBe Antibody

Blank well (Bw): OD_{BW} < 0.100;
Negative Control (NC): OD_{NC} value after blanking > 1.000; CV% < 10%
Antibody Calibrator (AC): $OD_{AC} < OD_{NC}/1.5$;
Positive Control (PC): $OD_{PC} < OD_{NC}/10$.

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

TROUBLESHOOTING

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

HBe Antigen

Problem	Check
Blank well: OD_{450nm} > 0.100	1. that the Chromogen/Substrate solution has not become contaminated during the assay.
Negative Control: OD_{450nm} after blanking > 0.150 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 the negative one); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to spills of positive samples or of 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 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.
Positive Control: OD_{450nm} < 1.500	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: OD_{450nm} > 0.100	1. that the Chromogen/Substrate solution has not become contaminated during the assay.
Negative Control: OD_{450nm} after blanking < 1.000 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 (dispensation of positive control instead of the negative one); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to spills of positive samples or of 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: $OD_{450nm} > OD_{NC}/1.5$	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of Negative Control instead of Calibrator, 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: $OD_{450nm} > OD_{NC}/10$	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.

RESULTS

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

HBe Antigen

Cut-Off (Co) = NC + 0.100

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

HBe Antibody

$$\text{Cut-Off (Co)} = \frac{(NC + PC)}{3}$$

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.

INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD_{450nm} (S) and the Cut-Off value (Co), mathematically S/Co and Co/S, according to the following tables:

HBe Antigen

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

HBe Antibody

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

An example of calculation of HBeAg assay is reported below.

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

Negative Control:	*0.020 – 0.030 – 0.025 OD_{450nm}*
Mean Value:	*0.025 OD_{450nm}*
Lower than 0.150	*Accepted*
Positive Control:	*2.489 OD_{450nm}*
Higher than 1.500	*Accepted*
Cut-Off =	*0.025+0.100 = 0.125*
Calibrator:	*0.520 - 0.540 OD_{450nm}*
Mean value:	*0.530 OD_{450nm}*
S/Co:	*4.2*
S/Co higher than 2.0	*Accepted*
Sample 1:	*0.030 OD_{450nm}*

Sample 2: *1.800 OD_{450nm}*
Sample 1 S/Co *< 0.9; negative*
Sample 2 S/Co *> 1.1; positive*

An example of calculation for HBeAb assay is reported below.

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

Negative Control: *2.100 – 2.200 – 2.000 OD_{450nm}*
Mean Value: *2.100 OD_{450nm}*
Higher than 1.000 *Accepted*

Positive Control: *0.100 OD_{450nm}*
Lower than NC/10 *Accepted*

Cut-Off *(2.100 + 0.100) / 3 = 0.733*
Calibrator: *0.720-0.760 OD_{450nm}*
Mean value: *0.740 OD_{450nm}*
OD450nm < NC/1.5 *Accepted*

Sample 1: *0.020 OD_{450nm}*
Sample 2: *1.900 OD_{450nm}*
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.*

PERFORMANCE CHARACTERISTICS

HBe Antigen

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.

HBeAg/Ab 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)

Lot ID	1x	2x	4x	8x	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

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	Delta B. 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	Delta B. EIA	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

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%.

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:

HBeAg/Ab: 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

HBeAg/Ab: 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

HBeAg/Ab: 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

<u>HBe Antibody</u>

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.

HBeAg/Ab 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):

Lot ID	1x	2x	4x	8x	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

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	Delta B. EIA 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 an other commercial product.

Member	PEI U/ml	Delta B. EIA	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

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% .

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:

HBeAg/Ab: 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

HBeAg/Ab: 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

HBeAg/Ab: 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

LIMITATIONS OF THE PROCEDURE

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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