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IVD | See external label | 2°C–8°C | 96 tests | REF 1705-12

AccuDiag™ HBeAg ELISA

REF 1705-12

HEPATITIS B VIRUS E ANTIGEN ELISA

Test	**HBeAg ELISA**
Method	**ELISA: Enzyme Linked Immunosorbent Assay**
Principle	**Sandwich ELISA**
Detection Range	**Qualitative Positive; Negative**
Sample	**50µl Serum**
Specificity	**~99.89%**
Sensitivity	**100%**
Total Time	**~ 75 min**
Shelf Life	**12 Months from the manufacturing date**

*Read the package insert carefully and completely before performing the assay. Follow the instructions and do not modify them. Only by strict adherence to these instructions, the erroneous results can be avoided and the optimal performance of DAI ELISA achieved.

INTENDED USE

The Diagnostic Automation, Inc. (DAI) HBeAg ELISA is an enzyme-linked immunosorbent assay (ELISA) kit for the qualitative detection of hepatitis B virus e antigen (HBeAg) in human serum or plasma. It is intended for use in clinical laboratories for diagnosis and management of patients related to infection with the hepatitis B virus. “Export Use Only”

SUMMARY

Hepatitis B virus (HBV) is an enveloped, double- stranded DNA virus belonging to the Hepadnaviridae family and is recognized as the major cause of blood transmitted hepatitis together with hepatitis C virus (HCV). Infection with HBV induces a spectrum of clinical manifestations ranging from mild, inapparent disease to fulminant hepatitis, severe chronic liver disease, which in some cases can lead to cirrhosis and carcinoma of the liver. Classification of a hepatitis B infection requires the identification of several serological markers expressed during three phases (incubation, acute and convalescent) of the infection. Now several diagnostic test are used for screening, clinical diagnosis and management of the disease.

Hepatitis B “e” antigen is a virus protein to be intimately associated with hepatitis B virus replication, indicating high degree of infectivity. HBeAg appears shortly after HBsAg and is detectable for few days to several weeks. During treatment and recovery, the titer of HBeAg declines and is replaced by the corresponding antibody (anti-HBe). In chronic hepatitis B infections, elevated levels of HBeAg can be detected for years, which is a marker for large quantity of virus. In some chronic HBsAg positive patients, HBeAg is undetectable due to HBV mutations suggesting for low level of viral replication. If HBeAg is considered a specific marker of infectivity, the presence of anti-HBeAg antibody in blood is recognized to be a clinical sign of recovery from the infection.

PRINCIPLE OF THE ASSAY

The DAI HBeAg ELISA kit uses “sandwich” ELISA method in which, polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBeAg. Patient’s serum or plasma sample is added to the microwell together with a second monoclonal antibody conjugated to horseradish peroxidase (HRP-Conjugate). During incubation, the specific immunocomplex formed in case of presence of HBeAg in the sample is captured on the solid phase. After washing to remove sample and unbound HRP-Conjugate, Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added into the wells. In presence of the antibody-antigen-antibody(HRP) “sandwich” complex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antigen captured in the wells, and to the sample respectively. Wells containing samples negative for HBeAg remain colorless.

COMPONENT

Microwell plate (96 wells): Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. Each well contains monoclonal antibodies reactive to HBeAg. The microwell strips can be broken to be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2-8°C. Once open, stable for 4 weeks at 2-8°C.

Negative Control (1x1ml per vial): Yellowish liquid filled in a vial with green screw cap. Protein-stabilized buffer tested non-reactive for HBeAg. Ready to use as supplied. Once open, stable for 4 weeks at 2-8°C. Preserv.0.1% ProClin™ 300.

Positive Control (1x1ml per vial): Red-colored liquid filled in a vial with red screw cap. Recombinant, non-infective HBeAg diluted in protein-stabilized buffer. Ready to use as supplied. Once open, stable for 4 weeks at 2-8°C. Preserv.0.1% ProClinTM 300.

HRP-Conjugate (1x6.5ml per vial): Red-colored liquid in a white vial with red screw cap. Horseradish peroxidase-conjugated anti-HBe. Ready to use as supplied. Once open, stable for 4 weeks at 2-8°C. Preserv.0.1% ProClinTM 300.
Wash Buffer (1x30ml per vial): Dilute before use! Colorless liquid filled in a clear bottle with white screw cap. Buffer solution containing surfactant. The concentrate must be diluted 1 to 20 with distilled/ deionized water before use. Once diluted, stable for 1 week at room temperature, or for 2 weeks when stored at 2-8°C. Detergent Tween-20.
Chromogen Solution A (1x7ml per vial): Colorless liquid filled in a white vial with green screw cap. Urea peroxide solution. Ready to use as supplied. Once open, stable for 4 weeks at 2-8°C.
Chromogen Solution B (1x7ml per vial): Colorless liquid filled in a black vial with black screw cap.TMB (Tetramethyl benzidine) solution. Ready to use as supplied. Once open, stable for 4 weeks at 2-8°C.
Stop Solution (1x7ml per vial): Colorless liquid in a white vial with white screw cap. Diluted sulfuric acid solution (0.5M H2SO4). Ready to use as supplied. Once open, stable for 4 weeks at 2-8°C.
Plastic sealable bag (1 unit): For enclosing the strips not in use
Package insert (1copy)
Cardboard plate cover (1sheet): To cover the plates during incubation and prevent evaporation or contamination of the wells

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Freshly distilled or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing system and/or pipette, disposable pipette tips, absorbent tissue or clean towel, dry incubator or water bath, 37±1°C, plate reader, single wavelength 450nm or dual wavelength 450/630nm, microwell aspiration/wash system.

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

1. Specimen Collection: No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the specimen should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, but highly lipaemic, icteric, or hemolytic specimens should not be used as they can give false results in the assay. Do not heat inactivate specimens. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.
3. DAI HBeAg ELISA is intended ONLY for testing of individual serum or plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
4. Transportation and Storage: Store specimens at 2-8°C. Specimens not required for assaying within 1 week should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

SPECIAL INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles with dispensing of 350-400**µ**l/well, are sufficient to avoid false positive reactions and high background (all wells turn yellow).
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation, do not

discard the content of the wells, but allow the plate washer to aspirate it automatically.
4. Assure that the microplate washer's liquid dispensing channels are not blocked or contaminated, and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to perform at least **5 washing cycles**, dispensing 350-400 **µ**l/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution (final concentration of 2.5%) for 24 hours, before liquids are disposed in an appropriate way.
7. The concentrated Washing solution should be diluted **1 to 20** before use. If less than a whole plate is used, prepare the proportional volume of solution.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8° C; **do not freeze**. To assure maximum performance of the DAI HBeAg ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

FOR PROFESSIONAL USE ONLY

The ELISA assays are time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature (18-30° C) before use. Shake reagent gently before, and return to 2-8° C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
7. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
8. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations.
9. Assure that the incubation temperature is 37° C inside the incubator.
10. When adding samples, avoid touching the well's bottom with the pipette tip.
11. When measuring the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
12. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.
13. If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

14. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
15. WARNING: Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
16. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
17. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
18. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.
19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.
20. The Stop solution 0.5M H_2SO_4 is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.
21. ProClin™ 300 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

INDICATION OF STABILITY DETERIORATION OF THE REAGENT

Values of the Positive or Negative controls, which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one or contact DAI technical support for further assistance.

ASSAY PROCEDURE

Reagents preparation: Allow the reagents to reach room temperature (18-30°C). Check the Wash buffer concentrate or the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and only clean vessels to dilute the buffer. All other reagents are READY TO USE AS SUPPLIED.

1. **Preparation:** Mark three wells as Negative control (e.g. B1, C1, D1), two wells as Positive control (e.g. E1, F1) and one Blank (e.g. A1, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

2. **Adding Sample:** Add 50µl of Positive control, Negative control, and Specimen into their respective wells except the Blank. Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination. Mix by tapping the plate gently.

3. **Adding HRP-Conjugate:** Add 50µl of HRP-Conjugate into each well except the Blank, and mix by tapping the plate gently.

4. **Incubating:** Cover the plate with the plate cover and incubate for 60 minutes at 37°C.

5. **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Washing buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders

6. **Coloring:** Add 50µl of Chromogen A and 50µl Chromogen B solutions into each well including the Blank. Incubate the plate at 37°C for 15 minutes avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and HBeAg positive sample wells.

7. **Stopping Reaction**: Using a multichannel pipette or manually, add 50µl of Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and HBeAg positive sample wells.

8. **Measuring the Absorbance**: Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 10 minutes after stopping the reaction).

INTERPRETATION OF RESULTS AND QUALITY CONTROL

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample's optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

1. **Calculation of Cut-off value (C.O.) = *Nc × 2.1**
***Nc** = the mean absorbance value for three negative controls.
Important: If the mean OD value of the negative control is lower than 0.05, take it as 0.05.

2. Quality control (assay validation):
The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

- The A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450 nm.
- The A values of the Positive control must be ≥ 0.800 at 450/630nm or at 450nm after blanking.
- The A values of the Negative control must be ≤ 0.100 at 450/630nm or at 450nm after blanking.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded and the mean value calculated again using the remaining two values. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Example:

Quality Control
Blank well A value: A1= 0.025 at 450nm (Note: blanking is required only when reading with single filter at 450nm)

Well No.: B1 C1 D1
Negative control A values after blanking: 0.020 0.012 0.016

Well No.: E1 F1
Positive control A values after blanking: 2.421 2.369

All control values are within the stated quality control range

Calculation of Nc: = (0.020+0.012+0.016)/3 = 0.016 (Nc is lower than 0.05, so take it as 0.05)

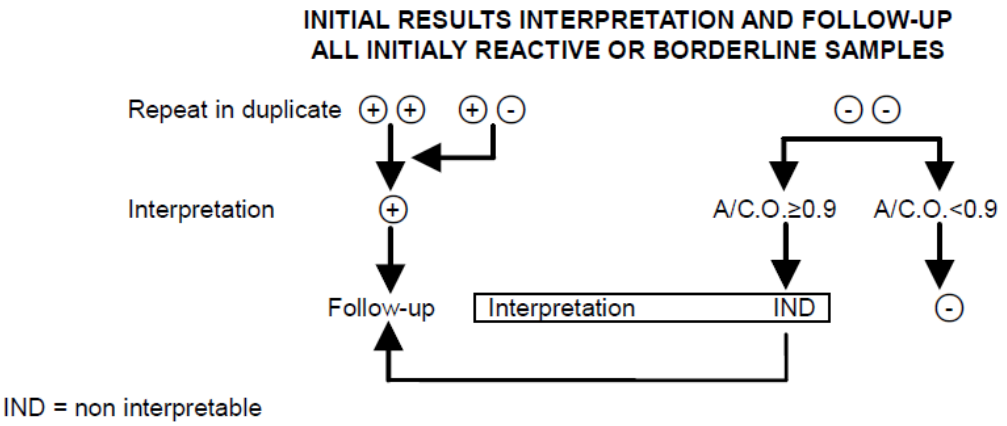
Calculation of the Cut-off: (C.O.) = 0.05 × 2.1 = 0.105

3. Interpretations of the results:
Negative Results (A / C.O. < 1): Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no HBeAg has been detected with DAI HBeAg ELISA. This result should not be used alone to stablish the infection state.

Positive Results (A / C.O. ≥ 1): Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that HBeAg has probably been detected using DAI HBeAg ELISA. All initially reactive specimens should be retested in duplicates using DAI HBeAg ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for HBeAg with DAI HBeAg ELISA. However, any positive result should not be used alone to establish the infection state.

Borderline (A / C.O. = 0.9-1.1): Specimens with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results. Repeatedly reactive samples can be considered positive for HBeAg.

Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.



IND = non interpretable

- If, after retesting of the initially reactive samples, both wells are negative results (A/C.O.<0.9), these samples should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step. For more information regarding DAI ELISA Troubleshooting, please refer to DAI's "ELISAs and Troubleshooting Guide".
- If after retesting in duplicates, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for HBeAg.
- After retesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.

TEST PERFORMANCE CHARACTERICS

Clinical Specificity: This clinical specificity of this kit has been determinate by a panel of samples obtained from 4360 healthy blood donors and 150 undiagnosed hospitalized patients. The repeatedly reactive samples and samples confirmed positive with the reference test were not included in the calculation of specificity.

Clinical Sensitivity: The clinical sensitivity of this HBeAg ELISA kit was calculated by a panel of samples obtained from 813 hepatitis B patients with well-characterized clinical history based upon reference assays for detection of HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc. Licensed HBeAg ELISA was used as a confirmatory assay. The evaluation results are given below.

Analytical Specificity: No cross reactivity was observed with samples from patients infected with HAV, HCV, HIV, CMV, and TP. No interference from rheumatoid factors up to 2000U/ml and no high dose hook effect up to HBeAg concentrations of 150000NCU were observed. The assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein. Frozen specimens have been tested to check for interferences due to collection and storage.

Specificity	Samples	-	+	Confirmed positive	Specificity	False positive
Donors	4360	4346	14	9	99.86%	5
Patients	150	132	18	18	100%	0
TOTAL	4510	4478	32	32	99.93	5

Sensitivity	Samples	-	+	Confirmed positive	Sensitivity	False Negatives
Acute	378	172	206	206	100%	0
Chronic	347	162	185	185	100%	0
Recovery	88	63	25	25	100%	0
TOTAL	813	397	416	416	100%	0

LIMITATIONS

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of ELISA method. The kit is designed to achieve performance characteristics of very high sensitivity and specificity. However, in very rare cases, some HBV mutants or subtypes could remain undetectable. Antibodies may also be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
3. Any positive result must be interpreted in conjunction with patient clinical information and other laboratory testing results.
4. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
5. The prevalence of the marker will affect the assay's predictive values.
6. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

7. This is a qualitative assay and the results can not be used to measure antigens concentrations.

REFERENCES:

1. Dienstag J.L. Hepatitis A virus: identification, characterization and epidemiological investigations. Progress in liver disease VI, Popper E., Schaffner F. (Eds), pp. 343-370, New York, Gruner and Stratton, 1979.
2. Duermeyer W., Van der Veen J., Koster B.ELISA in hepatitis A. Lancet. i : 823-824, 1978.
3. J.V. PARRY, (1981). Hepatitis A infection: guidelines for development of satisfactory assays for laboratory diagnosis.The Institute of Medical Laboratory Sciences 38, 303-311.
4. Lindberg J., Frosner G., Hansson B.G. et al. Serologic markers of hepatitis A and B in chronic active hepatitis. Scandinavian Journal of Gastroenterology,13:525-527, 1978.

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