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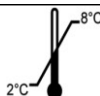
IVD	See external label		96 tests	REF 1778-12

AccuDiag™ HBcAb IgM

REF **1778-12**

Test	HBcAb IgM ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	ELISA - Indirect; Antigen Coated Plate
Detection Range	Qualitative Positive; Negative control & Cut off
Sample	100 µl Serum
Specificity	99.88%
Sensitivity	99.68%
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 Diagnostic Automation,Inc. HBcAb IgM ELISA achieved.*

INTENDED USE

The intended use for the Diagnostic Automation Inc. (DAI) **HBcAb IgM ELISA test** is for use in clinical laboratories for diagnosis and management of patients related to infection with hepatitis B virus. The HBcAb IgM ELISA is an enzyme-linked immunosorbent assay for the qualitative identification of IgM class antibodies to hepatitis B core antigen in human serum/plasma.

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 diseases, which in some cases can lead to cirrhosis and carcinoma of the liver. Classification of a hepatitis B infection requires the identification of a number of 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 "core" antigen (HBcAg) is a major component of the viral structure. HBcAg is composed of a single polypeptide of about 17 kD that is released upon disaggregation of the core particles; the antigen contains at least one immunological determinant. Antibodies to HBcAg (anti-HBc total antibody and IgM) appear shortly after the appearance of HBsAg and persist for life both in persons who have recovered from a hepatitis B infection and in those who develop HBsAg-carrier status but in rare cases, an HBV infection can also run its course without the appearance of immunologically detectable anti-HBc (usually in immunosuppressed patients).

In chronic hepatitis, however, spikes of anti-HBc IgM synthesis are present, confirming reactivation of HBV in hepatocytes and giving origin to permanent IgM low titers. Presence of IgM and total anti-HBc indicates an ongoing or recent HBV infection. When used in conjunction with tests for other HBV serological markers, a laboratory diagnosis or a rule out of HBV infection can be achieved.

PRINCIPLE OF THE ASSAY

This kit is a two-step incubation, solid phase antibody capture ELISA assay, in which polystyrene microwell strips are pre-coated with antibodies directed to human immunoglobulin M proteins (anti-μ chain). The patient's serum/plasma sample is added and during the first incubation step, any IgM-class antibodies will be captured inside the wells. After washing out all other components of the sample and in particular IgG-class antibodies, the specific anti-HBc IgM captured on the solid phase is detected by the addition of purified HBcAg, labeled with anti-HBc monoclonal antibody conjugated to horseradish peroxidase (HRP-Conjugate). During the second incubation, the HRP-conjugated antigens will specifically react only with anti-HBc IgM antibodies, and after washing to remove the unbound HRP-conjugate, Chromogen solutions are added to the wells. In presence of the (anti-μ chain)-(anti-HBc IgM)-(HBcAg-Ab (HRP)) immunocomplex, 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 antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HBc IgM remain colorless.

COMPONENTS

Σ 96 Tests

- **MICROWELL PLATE** **1 plate**
  Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant.
  8×12/12×8-well strips per plate.
  Each well contains anti-IgM antibodies (anti-μ chain).
  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** **1 vial**
  Yellowish liquid filled in a vial with green screw cap.
  0.5 ml per vial.
  Protein-stabilized buffer tested non-reactive for anti-HBc IgM.
  Preservatives: 0.1% ProClin 300. Ready to use as supplied.
  Once open, stable for 4 weeks at 2-8°C.

- **POSITIVE CONTROL** **1 vial**
  Red-colored liquid filled in a vial with red screw cap.
  0.5ml per vial.
  Anti-HBc IgM antibodies diluted in protein-stabilized buffer.
  Preservatives: 0.1% ProClin 300. Ready to use as supplied.
  Once open, stable for 4 weeks at 2-8°C.

- **HRP-CONJUGATE REAGENT** **1 vial (12 ml)**
  Red-colored liquid filled in a white vial with red screw cap.
  Horseradish peroxidase-conjugated purified HBcAg, labeled with monoclonal anti-HBc.
  Ready to use as supplied.
  Once open, stable for 4 weeks at 2-8°C.

- **WASH BUFFER** **1 bottle**
  Colorless liquid filled in a white bottle with white screw cap.
  50 ml per bottle.
  pH 7.4, **20x** PBS (Contains Tween-20 as a detergent).
  The concentrate must be diluted **1 to 20** with distilled/deionized water before use. Once diluted, stable for one week at room temperature, or for two weeks when stored at 2-8˚C.

- **CHROMOGEN SOLUTION A** **1 vial**
  Colorless liquid filled in a white vial with green screw cap.
  7ml per vial.
  Urea peroxide solution.
  Ready to use as supplied.
  Once open, stable for 4 weeks at 2-8°C.

- **CHROMOGEN SOLUTION B** **1 vial**
  Colorless liquid filled in a Black vial with Black screw cap.

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7ml per vial.
TMB (Tetramethylbenzidine) solution.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **STOP SOLUTION** **1 vial**
  Colorless liquid filled in a white vial with yellow screw cap.
  7ml per vial.
  Diluted sulfuric acid solution (0.5M H_2SO_4).
  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.

- **CARDBOARD PLATE COVER** **2 sheets**
  To cover the plates during incubation and prevent evaporation or contamination of the wells.

- **PACKAGE INSERT** **1 copy**

MATERIALS AND INSTRUMENTS REQUIRED BUT NOT PROVIDED

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
5. Absorbent tissue or clean towel.
6. Dry incubator or water bath, 37±0.5°C.
7. Microwell prate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
8. 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 hemolysis 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 lipemic, 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 HBcAb-IgM 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 7 days 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.

5. **Specimen Preparation:** Each specimen must be diluted 1:1000 with normal saline.

SPECIAL INSTRUCTIONS FOR WAHSING

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 of 350-400μl/well are sufficient to avoid false positive reactions and high background.
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 cycles, dispensing 350-400μl/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 they are disposed of 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.

PRECAUTIONS AND SAFETY

This kit is intended **FOR IN VITRO USE ONLY**

FOR PROFESSIONAL USE ONLY

The ELISA assay is a time and temperature sensitive method. 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 for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. **CAUTION - CRITICAL STEP**: Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 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 the 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. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.
9. Assure that the incubation temperature is 37°C inside the incubator.
10. When adding specimens, do not touch the well's bottom with the pipette tip.
11. When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.
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.

INDICATIONS OF INSTABILITY 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 Diagnostic Automation, Inc. technical support for further assistance.

- ProClin 300, Sphrases: S26-28-36/37/39-45-60-61, R phrases: 43
- Do not eat and drink at the laboratory
- Ware protective clothing and eye protection
- Biohazard: danger

ASSAY PROCEDURE

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) **Diluting Sample**: Dilute each specimen 1:1000 with normal saline. Do not dilute the Controls, as they are ready for use as supplied.
3) **Adding Sample**: Add 100µl of Positive control, Negative control, and Specimen into their respective

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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.

4) **Incubating**: Cover the plate with the plate cover and incubate for 30 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 Wash 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) **Adding HRP-Conjugate**: Add 100μl of HRP-Conjugate into each well except the Blank.
7) **Incubating**: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.
8) **Washing**: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash 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.
9) **Coloring**: Add 50μl of Chromogen A and 50μl of 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 anti-HBc IgM positive sample wells.
10) **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 anti-HBc IgM positive sample wells.
11) **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).

CALCULATION OF RESULTS AND QUALITY CONTROL

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) 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 A value from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and controls.

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

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 should be calculated by 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:

1. 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:	2.363	2.436	

All control values are within the stated quality control range

2. Calculation of Nc: $= \frac{(0.020+0.012+0.016)}{3} = 0.016$ (Nc is lower than 0.05, so take it as 0.05)

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

INTERPRETATION OF RESULTS

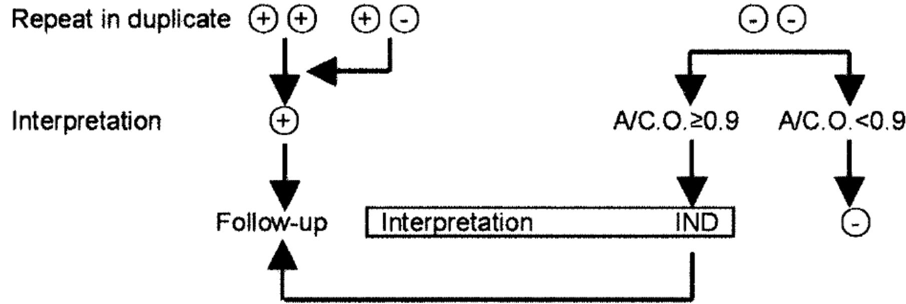
Negative Results (A / C.O. < 1): Specimens giving A value less than the Cut-off value are negative for this assay, which indicates that no IgM-class antibodies to hepatitis B core antigen have been detected with DAI HBcAb-IgM ELISA, there are no serological evidences for recent infections with HBV and the patient is probably not infected with the virus.

Positive Results (A / C.O. ≥ 1): Specimens giving A value equal to or greater than the Cut-off value are considered initially reactive, which indicates that IgM-class antibodies to hepatitis B core antigen have probably been detected with DAI HBcAb-IgM ELISA. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for anti-HBc IgM. Positive results with anti-HBc IgM detection indicate possible recent infection with HBV.

Borderline (A / C.O. = 0.9-1.1): Specimens with A value 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.

Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system (e.g. PCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings. The result from this assay should not be used alone to establish the infection state.

INITIAL RESULTS INTERPRETATION AND FOLLOW-UP
ALL INITIALLY REACTIVE OR BORDERLINE SAMPLES



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.
- 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 HBcAb IgM.
- 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.

PERFORMANCE CHARACTERISTICS

The **clinical specificity** of this assay has been determinate by a panel of samples obtained from 2500 healthy blood donors and 230 undiagnosed hospitalized patients. The repeatedly reactive samples and samples confirmed positive with the reference test were not included in the calculation of specificity.

Specificity	Samples	-	+	Confirmed positive	Specificity	False pos.
Donors	2500	2492	8	5	99.88%	3
Patients	230	210	20	20	100%	0
TOTAL	2730	2702	28	25	99.89%	3

The **clinical sensitivity** of this anti-HBc IgM ELISA kit has been calculated by a panel of samples obtained from 548 hepatitis B patients with well-characterized clinical history based upon reference assays for detection of HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc. This panel included samples from acute, chronic and recovered hepatitis B patients. Licensed anti-HBc IgM ELISA test was used as a confirmatory assay. The evaluation results are given below. Results obtained in individual laboratories may differ.

Sensitivity	Samples	-	+	Confirmed positive	Sensitivity	False neg.
Acute	318	4	314	315	99.68%	1
Chronic	128	110	18	18	100%	0
TOTAL	446	114	332	333	99.70%	1
Recovery	102	101	1	1	100%	0

Marker prevalence in follow up of patients infected with HBV:

Days Since infection	Number of samples	+	-	Detected prevalence of anti-HBc IgM

0	10	2	8	20%
1-10	12	3	9	25%
11-20	13	4	9	30%
21-30	9	8	1	88%
31-50	9	9	0	100%
51-70	14	14	0	100%
71-100	11	11	0	100%
101-120	8	8	0	100%
121-150	3	3	0	100%
151-170	2	1	1	50%
171-200	1	0	1	0%
Total:	92	63	29	68.48%

Analytical Specificity:

1. No cross reactivity observed with samples from patients infected with HAV, HCV, HIV, CMV, TP, and HTLV.
2. No interferences from rheumatoid factors up to 2000U/ml were observed during clinical testing. The assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein.
3. Frozen specimens have been tested to check for interferences due to collection and storage.

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 DAI HBcAb IgM ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

LIMITATIONS

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immunosuppressed individuals. Therefore, negative results obtained with DAI HBcAb-IgM ELISA are only indication that the sample does not contain detectable level of anti-HBc IgM.
3. 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.
4. The most common assay mistakes are: using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
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 kit is a qualitative assay and the results cannot be used to measure antibodies concentrations.

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SUMMARY OF THE ASSAY PROCEDURE:	
Dilute sample with normal saline	**1:1000**
Add sample / Controls	**100µl**
Incubate	**30 minutes**
Wash	**5 times**
Add HRP-Conjugate	**100 µl**
Incubate	**30 minutes**
Wash	**5 times**
Coloring	**50µl A + 50µl B**
Incubate	**15 minutes**
Stop the reaction	**50µl stop solution**
Read the absorbance	**450nm or 450/630 nm**

Date Adopted	**2017-03-16**
1778-12	**DAI HBcAb IgM ELISA**

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