bi-x-act® of FINLAND

Anti-HBc IgM ELISA 96 Well Plate Test Kit

CE IVD

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1) Intended Use

Anti-HBc IgM ELISA Test is an enzyme immunoassay for in vitro qualitative detection of IgM antibody to hepatitis B virus core antigen (Anti-HBc IgM) in human serum or plasma (heparin, EDTA or citrate).

2) Summary and Test Explanation

The hepatitis B virus (HBV) consists of an external envelope (HBsAg) and an inner core (HBcAg). In acute HBV infection, IgM antibodies to HBcAg (Anti-HBc IgM) are detectable in serum or plasma shortly after the onset of viral replication and remain in the circulation for about 7 to 17 weeks. The detection of anti-HBc IgM antibodies is used, in conjunction with HBsAg determination, as indicative marker of the phase of the infection and for the monitoring of patients under treatment with interferon. High anti-HBc IgM titers can be found in acute HBV infection and in attacks during chronic hepatitis B. The level of anti-HBc IgM decreases throughout the course of infection. However, low levels of anti-HBc IgM may persist for over a year after infection in some patients and are found occasionally in chronic carriers**.**[*1-6]

Anti-HBc IgM ELISA Test is a fast test for the qualitative detection of the presence of IgM antibodies to HBcAg in serum or plasma (heparin, EDTA or citrate) specimens. The test utilizes Anti-human IgM on microtiter wells as solid phase and HBcAg and peroxidase-conjugated Anti-HBc in liquid phase in an "IgM capture" principle to detect Anti-HBc IgM levels in serum or plasma.
Specimens with absorbance values ≤ 0.9 x signal/cutoff ratio are considered **NON-REACTIVE** for Anti-HBc IgM. Specimens with absorbance values ≥ 1.1 x signal/cutoff ratio are considered **REACTIVE** for Anti-HBc IgM.
If the signal/cut-off ratio is within Retest Range (0.9 - 1.1), the test must be repeated in duplicate and interpreted as above.

3) 3) Test Description

Anti-HBc IgM ELISA Test is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immunosorbent assay) –- based on the principle of **"Anti-HBc IgM"**. The solid phase of the microtiter plate is made of polystyrene wells coated with anti-human IgM.
When a serum or plasma specimen containing Anti-HBc IgM is added to the Anti-human IgM-coated wells and incubated, IgM antibodies present in the specimen bind to the Anti-h IgM on the wells. After addition of an HBcAg-containing reagent and a solution containing peroxidase-conjugated anti-HBc a further incubation takes place, during which (Anti-h IgM) • (Anti-HBc IgM) •(HBcAg) • (Anti-HBc• peroxidase) complex is formed on the wells. After washing the

microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. If Anti-HBc IgM is present in the specimen, after washing, the activity of peroxidase on the wells reflects the content of anti-HBc IgM in a specimen. The peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450 nm with a selected reference wavelength within 620 to 690 nm[*8]

The above described test principle is shown also in the following figure.

A. Specimen (containing human IgM Anti-HBc):

1. Plate (Anti-h IgM) + specimen (containing Anti-HBc IgM) -→ plate (Anti-h IgM) • Anti-HBc IgM
2. Plate (Anti-h IgM)• Anti-HBc IgM + HBcAg + Anti-HBc • peroxidase → plate (Anti-h IgM) • Anti- HBc IgM • HBcAg • (Anti-HBc• peroxidase) complex
3. Wash to remove the unbound materials.
4. Plate (Anti-h IgM) • IgM Anti-HBc IgM • HBcAg • (Anti-HBc • HRPO) complex + TMB substrate solution → light blue to blue color
5. Light blue to blue color + sulfuric acid →light yellow to yellow color, measured at 450nm with a selected reference wavelength within 620 to 690nm[*8]

B. Specimen (without IgM Anti-HBc):

1. Plate (Anti-h IgM) + specimen (without Anti-HBc IgM) → plate (Anti-h IgM)
2. Plate (Anti-h IgM) + HBcAg + Anti-HBc • peroxidase → plate (Anti-h IgM)------ no complex will form
3. Wash to remove the unbound materials.
4. Plate (Anti-human IgM) + TMB substrate solution (colorless) → colorless
5. colorless + sulfuric acid →colorless, measured at 450nm with a selected reference wavelength within 620 to 690nm[*8]

4) Description of Materials Provided & Product Code System

●Item 1 - 8 on the following reagent table should be refrigerated at + 2 to +8°C . Washing Solution D (20X) and 2N H_2SO_4 can be stored at +2 to +30°C.

ITEMS	Components	Description	Qt. per 96 tests
(1)	Anti-IgM Microtiter Plate	One microtiter plate (removable strips) with 96 wells coated with Anti-human IgM.	1 plate
(2)	Anti-HBc • Peroxidase Solution	Anti-HBc (human) • peroxidase (horseradish) conjugate in buffer with protein stabilizer. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1bottle, 7 ml
(3)	Anti-HBc IgM Positive Control	Human Anti-HBc IgM in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1 bottle, 2 ml
(4)	Specimen Diluent	Buffer containing protein stabilizers. Preservatives: 0.003%Gentamycin and 0.01% Thimerosal.	2 bottle 35 ml
(5)	HBcAg Reagent	HBcAg in buffer containing protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1 bottle, 7 ml
(6)	Anti-HBc IgM Negative Control	Normal (Anti-HBc IgM negative) human serum containing protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1 bottle, 2 ml
(7)	TMB Substrate Solution A	0.6 mg/ml of 3,3',5,5'-tetramethylbenzidine (TMB) in an organic base.	1 bottle, 10 ml
(8)	TMB Substrate Solution B	Citric acid buffer containing 0.03% H_2O_2.	1 bottle, 10 ml

(9)	Conc. Washing Solution D (20x)	Phosphate buffer with Tween-20.	1 bottle 52 ml
(10)	2N H_2SO_4	2N H_2SO_4 (Sulfuric acid)	1 bottle 12 ml

● ACCESSORIES: (provided as needed)

ITEMS	Components
(11)	Adhesive Slips
(12)	Absorbent Pads
(13)	Black Cover

● OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

ITEMS	Components
(1)	5µl, 50µl and 100 µl micropipettes and tips are needed
(2)	Incubator or waterbath with temperature control at +37 ±1°C
(3)	Tubes for specimen dilution.
(4)	Plate washing equipment.
(5)	Purified water: distilled or deionized water.
(6)	ELISA microwell reader: Dual wavelength 450nm with 620-690nm as reference wavelength[*8], bandwidth 10nm.
(7)	Fully automatic EIA micro-plate analyzer is optional. User should validate the automatic EIA micro-plate analyzer in combination with the kit.

4.1) Storage Conditions and Stability of Kit and Components

Kit/components	Storage condition	State	Stability
Anti-HBc IgM ELISA Test Plate	+2～+8 °C	Original	15 months
		Once open	1 month
Anti-HBc IgM Positive Control	+2～+8 °C	Original	15 months
		Once open	1 month
Anti-HBc IgM Negative Control	+2～+8 °C	Original	15 months
		Once open	1 month
HBcAg Reagent	+2～+8	Original	15 months

	°C	Once open	1 month
Specimen Diluent	+2～+8 °C	Original	16 months
		Once open	1 month
Anti-human IgM Plate	+2～+8 °C	Original	15 months
		Once open	2 months
Anti-HBc • Peroxidase Conjugate Solution	+2～+8 °C	Original	15 months
		Once open	1 month
Concentrated Washing Solution (20x)	Room temp.	Original	24 months
		Once open	1 month
20x Diluted Washing Solution	Room temp.	Diluted	2 days
	+2～+8 °C	Diluted	1 week
TMB Substrate Solution A	+2～+8 °C	Original	18 months
		Once open	1 month
TMB Substrate Solution B	+2～+8 °C	Original	18 months
		Once open	1 month
2N Sulfuric Acid	Room temp.	Original	24 months
		Once open	1 month

5) Instruction for Use

5.1) Warnings:

5.1.1) This reagent kit is for professional use only.

5.1.2) This reagent kit is for *in vitro* diagnostic use only.

5.1.3) Bring all kit reagents and samples to room temperature (+20 to +30 °C) and mix carefully before use.

5.1.4) Do not use reagent beyond its expiration date.

5.1.5) Do not interchange reagents between different lots.

5.1.6) Do not pipette in the mouth.

5.1.7) Do not smoke or eat in areas where specimens or reagents are handled.

5.1.8) The positive control, negative control, HBcAg Reagent, conjugate solution and specimens should be regarded as potential hazards to health. They should be used and discarded according to the user's laboratory safety procedures. Such safety procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols.

5.1.9) Potential infectious specimens and non-acid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with the laboratory's practice for potential bio-hazard control.

5.1.10) Prior to dispose the waste of used specimens and kit reagents as general waste, it should be treated in accordance with the local procedures for potential bio-hazardous waste or treated as follows:

Both liquid and solid waste should be autoclaved maintaining +121 °C for at least 30 minutes.

Solid waste can also be incinerated.

Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%.

Acidic liquid wastes must be neutralized before treatment with sodium hypochlorite as mentioned above and should stand for 30 minutes to obtain effective disinfection.

5.1.11) 2N sulfuric acid is an irritant to skin, eyes, respiratory tract and mucous membranes. Avoid contact of the 2N sulfuric acid with skin and mucous membranes. In case of contact, clean with large lots of water immediately. In case of inhalation, supply fresh air and seek medical advice in case of complaints.

5.1.12) TMB substrate solution A contains organic solvent, which is flammable. TMB substrate solution A contains dimethyl sulfoxide, an irritant to skin and mucous membranes.

5.1.13) Although all human sourced material are tested non-reactive for Anti-HCV and Anti-HIV, and inactivated at +56 °C for one hour, the reagent shall be handled as potential infectious material.[*7]

5.2) Specimen Collection and Preparation for Analysis

5.2.1) No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques.

5.2.2) Either serum or plasma can be used with this diagnostic kit.

Whole blood specimen should be separated as soon as possible in order to avoid hemolysis. Any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen should be removed prior to use.

5.2.3) Specimens must be stored at +2 to +8 °C and avoided heat-inactivation to minimize deterioration. For long-term storage, specimens should be frozen below -20 °C. Storage in self-defrosting freezers is not recommended.

5.2.4) Frozen specimens must be thoroughly thawed and mixed homogenously before test.

5.2.5) Avoid multiple freeze-thaw procedures

5.2.6) **WARNING**

1. The specimen must not contain any compounds of AZIDE, which inhibits the peroxidase activity.
2. Incompletely coagulated serum samples and microbial-contaminated specimens should not be used.

5.3) Reagents Storage

5.3.1) The kit must be stored at + 2 to +8 °C. Do not freeze.

5.3.2) Strips of the plate should be used within 2 months after opening the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and taped the opening tightly.

5.3.3) Return reagents to +2 to +8 °C immediately after use.

5.3.4) Washing Solution D (20x) Concentrate is stored and shipped at +2 to +8 °C, which can cause crystallization. If crystal has been precipitated before use, warm up the solution in +37 °C water bath till the crystal is dissolved.

5.4) Plate washing procedure

5.4.1) Preparation of washing solution:

Dilute Washing Solution D (20x) Concentrate with distilled or de-ionized water to 1:20 dilution. Do not use tap water.

5.4.2) Plate washing:

(a) For plate washer with overflow aspirating function: 6 cycles with at least 0.5ml washing buffer per well per cycle

or

(b) For plate washer without overflow aspirating function: 8 cycles with at least 0.35ml washing buffer per well per cycle.

5.4.3) Blot dry by inverting the plate and tapping firmly onto absorbent paper. Too much residual wash buffer will cause false results.

WARNING

Improper washing will cause false results.

5.5) Test Procedure

5.5.1) Bring all reagents and specimens to room temperature (+20 to +30 °C) before assay. Adjust water bath or incubator to +37±1 °C.

5.5.2) Make 1+100 dilution of each specimen:

Prepare for each specimen a tube for dilution, with exception of the controls. Add 500 µl of Specimen Diluent and 5 µl of each specimen to each tube respectively and shake to mix.

NOTE:

a) Do not dilute the controls.

b) Use a new pipette tip after each sampling to avoid cross-contamination.

5.5.3) Reserve one well for Blank. Add 100 µl of Negative Control to each three wells, 100 µl of Positive Control to each two wells, 100 µl of Specimen Diluent to each of the other reaction wells for test specimen.

5.5.4) Add 5 µl of each diluted specimen to each well containing Specimen Diluent, respectively.

5.5.5) Gently tap the plate.

5.5.6) Remove the protective backing of the adhesive slip and press it on the reaction plate, so that it is tightly sealed.

5.5.7) Incubate the plate at +37 °C for 1 hour.

5.5.8) At the end of the incubation period, remove and discard the Adhesive Slip and wash plate by following "5.4. PLATE WASHING PROCEDURE".

5.5.9) Add 50 µl of HBcAg reagent to each reaction well except the Blank followed by 50 µl of Anti-HBc-Peroxidase solution. Apply a new adhesive slip.

5.5.10) Incubate the plate at +37 ± 1°C °C for 1 hour.

5.5.11) At the end of the incubation period, remove and discard the adhesive slip, wash the plate by following "5.4. PLATE WASHING PROCEDURE".

5.5.12) Select one of the following two methods for color development:

A. Mix equal volumes of **TMB Substrate Solution A and B** in a clean container immediately prior to use. Add **100 µl** of the mixture solution to each well including the blank well.

B. Add **50 µl** of **TMB Substrate Solution A** first, and then add **50 µl** of **TMB Substrate Solution B** into each well including the blank. Mix well gently .

NOTE: TMB Substrate Solution A should be colorless to light blue; otherwise, it should be discarded. The mixture of TMB

Substrate Solution A and B should be used within 30 minutes after mix. The mixture should be protected from exposition to intense light.

5.5.13) Cover the plate with the black cover and incubate at room temperature for 30 minutes.

5.5.14) Stop the reaction by adding 100 µl of 2N H_2SO_4 to each well including the blank.

5.5.15) Determine the absorbance of controls and test specimens within 15 minutes with a precision photometer at 450 nm with a selected reference wavelength within 620 to 690nm[*8].

Use the blank well to blank the photometer.

NOTE: The color of the blank should be colorless to light yellowish; otherwise, the test result is invalid. In this case the tests must be repeated.

Substrate blank : absorbance value must be less than 0.100.

5.6) Calculation of Test Results

5.6.1) Calculation of the NCx (Mean Absorbance of Negative Control).

Example:

Sample No.	Absorbance
1	0.068
2	0.072
3	0.070

NCx = (0.068+0.072 + 0.070) / 3 = 0.070

NOTE: NCx **must be ≤ 0.1, otherwise, the test is invalid.**

5.6.2) Calculation of PCx (Mean Absorbance of Positive Control)

Example:

Sample No.	Absorbance
1	1.612
2	1.613

PCx = (1.612 + 1.613) /2 = 1.613

NOTE: PCx **must be ≥ 0.4 , otherwise, the test is invalid.**

5.6.3) Calculation of **P-N Value**

P-N = PCx - NCx

Example:

P - N = 1.613 – 0.070 = 1.543

NOTE: P - N Value must be ≥ 0.3, otherwise, the test is invalid.

5.6.4) Calculation of the **Cutoff Value**

Cutoff Value = NCx + (PCx)/4

Example:

Cutoff Value = 0.070 + (1.613)/4 = 0.473

5.6.5) Calculation of the **Retest Range**

Retest Range = Cutoff Value ±10%

Example: Cutoff Value = 0.473

Retest Range = (0.473 - 0.047) to (0.473 +0.047) = 0.426 to 0.520

5.7) Validity of Test Runs

5.7.1) **NCx must be ≤ 0.1, otherwise, the test is invalid.**

5.7.2) **PCx must be ≥ 0.4 , otherwise, the test is invalid.**

5.7.3) **P-N Value must be ≥ 0.3 , otherwise, the test is invalid.**

NOTE: Negative Control: absorbance value must be less than or equal to 0.100 after subtracting the blank.

5.8) Interpretation of Results

Specimens with signal/cut-off ratio≤ 0.9 are considered non-reactive for Anti-HBc IgM. Specimens with signal/cut-off ratio ≥ 1.1 are considered reactive for Anti-HBc IgM.

If the signal/cut-off ratio is within Retest Range (0.9 - 1.1), the test must be repeated in duplicate and interpreted as above. If both results are non-reactive the final result is non-reactive, if both results are reactive the final result is reactive. Any other combination is an indeterminate result. Testing of follow up samples and other hepatitis B serological markers should be taken into account in case of an indeterminate result.

NOTE:

Interpretation of Results: The result of an Anti-HBc IgM test should always be interpreted taking into account other hepatitis B serological and NAT markers as well as clinical symptoms.

5.9) Troubleshooting

If the result cannot be reproduced, a preliminary troubleshooting should be performed by checking the possibilities listed below:

5.9.1) Improper washing procedure.

5.9.2) Contaminated with positive specimen.

5.9.3) Wrong volume of sample, conjugate or substrate.

5.9.4) Contamination of well rim with conjugate.

5.9.5) Improper specimen such as hemolyzed serum or plasma, specimen containing precipitate and specimen not thoroughly mixed before use.

5.9.6) Wrong incubation time or temperature.

5.9.7) Obstructed or partial obstructed washer aspirate/dispense head and needles.

5.9.8) Insufficient aspiration.

5.10) Limitations and Interferences

5.10.1) This reagent kit is to be used for un-pooled human serum or plasma samples only. It cannot be used for testing of non-human serum or plasma samples.

5.10.2) The reagent kit has not been validated for use with cadaveric samples.

5.10.3) Non-repeatable false positive results may be obtained with any enzyme immunoassay kits, largely due to technical error either on the part of the operator or malfunction of apparatus used.

5.10.4) When Anti-HBc IgM test results are used to differentiate acute from non-acute HBV infections, the clinical history of the patient and the results of other markers (if available) shall be taken into account.

5.10.5) A negative Anti-HBc IgM result does not preclude the possibility of previous infection with HBV.

5.10.6) Potential interfering substances:

Potential interfering samples, i.e. samples with hyperlipemia, hemolysis, hyper-bilirubinemia, samples with monoclonal immunoglobulin components, samples containing elevated levels of autoimmune antibodies (rheumatoid factor-RF, antinuclear antibodies-ANA, or anti-mitochondrial antibodies-ANA) did not affect the test result with the present **Anti-HBc IgM ELISA Test Kit**

5.10.7) The anticoagulants heparin, EDTA and sodium citrate have no influence on the specificity of **Anti-HBc IgM ELISA Test** and can be used to obtain plasma samples for analysis with the present Anti-HBc IgM kit.

5.11) Performance Characteristics

5.11.1) Diagnostic Specificity

Characteristics of the samples	No. of sample	Negative Result Reference Assay	Negative Result Anti-HBc IgM ELISA Test
Clinical/hospitalized patients (HBV negative 'clinicals')	200	200	200
Blood donors (HBV/Anti-HBc negative 'donors')	200	198	200
Sample containing potentially interfering substances	50	50	50
Total	450	448	450
Diagnostic Specificity	-------	99.5%	**100%**

5.11.1.1) Potential interfering substances

Potential interferences with the **Anti-HBc IgM ELISA Test** assay were investigated.

For each potential interfering substance, at least two serum samples containing different amounts of the potentially interfering substance were mixed in fixed ratios of 10 + 0; 7 + 3; 5 + 5; 3 + 7; 0 + 10 with other serum samples containing increased Anti-HBc IgM levels but no interfering factors. The neat samples as well as the mixtures were analyzed.

In particular the specificity study included:

- lipemic (turbid) samples before and after high speed centrifugation
- hemolytic samples or hemolysate
- icteric samples (=hyperbilirubinemia)
- samples with monoclonal immunoglobulin components
- samples containing elevated levels of autoimmune antibodies (rheumatoid factor - RF, antinuclear antibodies –ANA, or antimitochondrial antibodies-AMA)

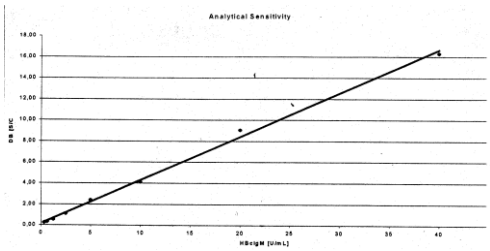
No interference was detected with both used lots, neither the type of anticoagulant had an influence on both tested lots of **Anti-HBc IgM ELISA Test**

5.11.2) Analytical Sensitivity and Linearity:

5.11.2.1) Detection limit using dilution of Anti-HBc IgM Reference Materials:

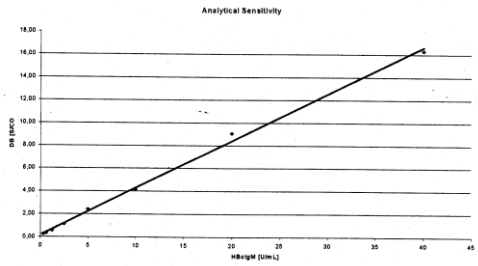
A serial dilutions of the Paul Ehrlich Institute (PEI) (Langen, Germany) Standard Material for Anti-HBc IgM (100 PEI U/ml) was used to evaluate the analytical sensitivity (detection limit) of Anti-HBc IgM ELISA Test **The analytical sensitivity (detection limit) was defined as the lowest concentration that can be detected.**
Both lots of Anti-HBc IgM ELISA Test had an analytical sensitivity at 2.5 PEI U/ml, better than the reference assay by one twofold dilution.

Analytical Sensitivity of Lot #B44333PT
S/C vs Concentration



PEI U/ml

Analytical Sensitivity of Lot #B44334PT
S/C vs Concentration



PEI U/ml

5.11.2.2) **Linearity using blood samples**
Linearity was evaluated with 2 lots of Anti-HBc IgM ELISA Test using high Anti-HBc-IgM positive serum samples after diluting them throughout the measuring range and then around the cutoff level in narrow dilution steps.

Linear Range: OD from 1.714 to 0.290
Linear Range: OD from 0.95 to 0.118
Linearity (semilog chart): R = 0.983
Linearity (semilog chart): R = 0.980

5.11.3) Diagnostic Sensitivity

Positive specimens/Specimens used to evaluate the diagnostic sensitivity/ Patients with HBV infection:

	Anti-HBc IgM ELISA Test	Reference Assay
positive	199	188
negative	1	13
indeterminate	7	6
total	207	207
Resolved diagnostic sensitivity	96.1%	90.8%

5.11.3.1) HBV infected individuals

207 known Anti-HBc-IgM positive samples were tested in **Anti-HBc IgM ELISA Test**, in which 199 of these 207 samples were detected as positive, 1 as negative and 7 as indeterminate.

The resolved diagnostic sensitivity for Anti-HBc IgM ELISA Test was 96.1 % (199/207) and better than the sensitivity of the CE marked reference assay, which showed a resolved diagnostic sensitivity of 90.8 % (188/207), detecting 188 of these 207 samples as positive, 13 as negative and 6 as indeterminate.

Samples	Anti-HBc IgM ELISA Test	Reference Assay
positive	199	188
negative	1	13
indeterminate	7	6
total	207	207
Resolved diagnostic sensitivity	96.1%	90.8%

5.11.4) Precision

5.11.4.1) Intra-run repeatability

For determination of intra-assay (within-run) precision, the positive control and two patient serum samples with different Anti-HBc IgM titer (slightly above the cutoff level and at medium level) were analyzed in replicates of 20 in a single "run" over 3 days and the measured absorbance were registered.

The mean and the within-run coefficient of variation (CV) for the positive control and patient sample were calculated.

Item tested	Sample size	precision
Positive Control	N = 20	CV ≤ 17.31%
Patient Serum #1	N = 20	CV ≤ 20.11%
Patient Serum #2	N = 20	CV≤ 13.53%

5.11.4.2) Inter-run reproducibility

Item tested	Sample size	precision
Positive Control	N = 60	CV ≤ 12.83%
Patient Serum #1	N = 60	CV ≤ 12.00%
Patient Serum #2	N = 60	CV ≤8.24%

5.11.5) Traceability

Concentration of Positive Control of Anti-HBc IgM ELISA Test = 640 PEI U/ml ±30%

5.11.6) Antibody Excess/High-dose Hook Effect

To check the antigen excess/high-dose hook effect of Anti-HBc IgM ELISA Test two serum samples with a very high – Anti-HBc IgM titer (OD≧1.5) were tested in serial dilution. No antibody excess/high-dose hook effect was observed in the two samples.

5.12) Flow Chart of the Test Procedure

Dilute 5 µl of each specimen with 500 µl of Specimen Diluent. Do not dilute controls.

↓

Add 100 µl controls (3x NC, 2x

PC) and add 100 µl Specimen Diluent into wells. Reserve one well for blank.

↓

Add 5µl of the Diluted specimen to each well containing Specimen Diluent. Sealed with adhesive slip.

↓

Incubate in +37 ±1 °C for 1 hour

↓

Wash the plate.

↓

Add 50 µl of HBcAg Reagent and 50 µl Anti-HBc Peroxidase Solution into each reaction well, except the blank.

↓

Incubate in +37 ±1 °C for 1 hour

↓

Wash the plate.

↓

(Choice one of the following two methods for color development)

↙ ↘

Mix equal volumes of TMB Substrate Solution A and B. Add 100 µl of the mixed solution to wells.

Add 50 µl of TMB Substrate Solution A to wells and then add 50 µl of TMB Substrate Solution B. Mix well, gently.

↘ ↙

Incubate at R.T. for 30 minutes.

↓

Add 100 µl of 2N H_2SO_4 into each well.

↓

Determine absorbance using 450 nm as reading wavelength with 620-690nm reference wavelength[*8]

6) Bibliography:

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6. Hadziyannis SJ, Hadziyannis AS, Dourakis S, et al. Clinical significance of quantitative anti-HBc IgM assay in acute and chronic HBV Infection. Hepatogastroenterology 1993;40:588–592.
7. Shikata T, Karasawa T, Abe K, et al. Incomplete inactivation of hepatitis B virus after heat treatment at +60°C for 10 hours, J. Infect. Dis. 1978;138:242-244.
8. The reference wavelength of spectrometer can be 620nm to 690nm. However, user should validate the photometer in combination with this kit before use.