IVD

MD2958

FOR PROFESSIONAL USE

Clonorchis-IgG-EIA-BEST

Enzyme immunoassay kit for the detection of IgG to Clonorchis sinensis antigens in blood serum (plasma)

INSTRUCTION MANUAL

1. INTENDED USE

"Clonorchis-IgG-EIA-BEST" kit is intended for the detection of immunoglobulins G (IgG) to *Clonorchis sinensis* antigens in human blood serum (plasma) using enzyme-linked immunosorbent assay (ELISA).

The kit can be used for the diagnosis of clonorchiasis along with other methods, in the study of pathogenesis, immunogenesis, and epidemiology of clonorchiasis in clinical and research laboratories.

The kit is designed to perform 96 tests including control samples: 12 independent ELISA runs, 8 samples each, including control samples, can be performed.

2. PRINCIPLE OF THE METHOD

The method of detection is based on the solid-phase enzyme-linked immunosorbent assay.

During the first incubation stage, specific antibodies to *Clonorchis* antigens (including IgG), if present in the test sample, interact with *Clonorchis* antigens immobilized on the surface of the plate wells.

During the second incubation stage, bound IgG to *Clonorchis* antigens interact with monoclonal antibodies to human IgG conjugated with horseradish peroxidase.

The amount of bound conjugate is determined using a color reaction with the peroxidase substrate – hydrogen peroxide and a chromogen – tetramethylbenzidine. After stopping the peroxidase reaction with Stop solution, the optical density of the solutions in the wells is measured at a primary wavelength of 450 nm and a reference wavelength of 620–655 nm. The intensity of coloring is proportional to the concentration of IgG to *Clonorchis* antigens in the test sample.

3. KIT CONTENTS

- Microplate (12 eight-well strips) coated with *Clonorchis* antigens – 1;
- Positive Control sample (C^+), contains IgG to *Clonorchis* antigens – 1 vial, 1.5 ml;
- Negative Control sample (C^-), free of IgG to *Clonorchis* antigens – 1 vial, 2.5 ml;

- Conjugate of monoclonal antibodies to human IgG with horseradish peroxidase – 1 vial, 13 ml;
- Phosphate buffered saline with tween, concentrate (PBS-T×25) – 1 vial, 28 ml;
- Solution for preliminary serum dilution (SPSD) – 1 vial, 10 ml;
- Serum diluent (SD) – 1 vial, 12 ml;
- Tetramethylbenzidine solution (TMB solution) – 1 vial, 13 ml;
- Stop solution – 1 vial, 12 ml.

The kit also includes:

- Adhesive film for strip sealing – 2;
- Plastic container for reagents – 2;
- Pipette tips – 16;
- Plate for pre-dilution of test samples – 1.

4. SPECIFICATIONS

4.1. Specificity. Cross-reactions are possible in case of opisthorchiasis, which may be caused either by co-invasion or by the interaction of antibodies with the heterologous antigen due to immunologic crosses between antigens.

4.2. Diagnostic sensitivity of IgG to *Clonorchis* antigens detection: clinical tests performed on 40 positive blood serum and samples showed 100% sensitivity (interval 91.2% – 100%, with a confidence level of 95%).

4.3. Diagnostic specificity of IgG to *Clonorchis* antigens detection: clinical tests performed on 40 negative blood serum samples showed 100% specificity (interval 91.2% – 100%, with a confidence level of 95%).

5. WARNINGS AND SAFETY PRECAUTIONS

Carefully follow the below-mentioned rules to ensure reliable results:

- For *in vitro* diagnostic use only.
- The kit must be used by skilled personnel only.

- Wear protective disposable gloves, laboratory coats, and eye protection when handling specimens and kit components.
- Stop solution and TMB solution have an irritating action. Caution should be observed to avoid spraying and adhering to skin and mucous membranes. If not avoided, wash the affected area with a large quantity of running water.
- Human blood serum/plasma used to prepare reagents of this kit was inactivated. Control samples and specimens should be handled as potentially infectious. Follow the National Safety Requirements when working with the kit.
- Do not use the components from the kits of different lots or mix them when preparing solutions, except for non-specific components (PBS-T×25, TMB solution, Stop solution) that are interchangeable throughout the entire range of kits produced by AO Vector-Best.
- Never use reagents from kits of other manufacturers with this kit.
- Use only disposable pipette tips. Never use the same pipette tip for different samples.
- Quality of washing of the wells is important for obtaining reliable accurate results. Use a plate washer for filling and aspirating the wells. Achieve complete filling and emptying of the wells in the washing process. Insufficient aspiration of fluids in the washing process may lead to a decrease in sensitivity and specificity of the analysis.
- Use disinfectants based on quaternary ammonium compounds, alcohols, or tertiary amines for glassware, avoid disinfectants containing active oxygen and chlorine.
- Always treat the working surface of the table and the cone of the pipette with 70% ethanol before starting the procedure. Do not use hydrogen peroxide or chloramine.
- Dispose of unused reagents and waste in accordance with country, federal, state, and local regulations.
- Do not use the kit after the expiration date.

– To obtain reliable results, strictly follow this Instruction Manual provided with the kit.

6. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

– Microplate reader equipped with 450/620–655 nm filters or automated open-type ELISA processor;

– Thermostat (37±1) °C;

– Refrigerator;

– Half-automatic variable-volume single-channel pipettes with disposable tips (5–5000 µl);

– Half-automatic variable-volume multi-channel pipettes with disposable tips (5–300 µl);

– Plate washer;

– 10–15 ml vials;

– Filter paper;

– Disposable medical non-sterile powder-free gloves;

– 1000 ml measuring cylinder;

– 1000 ml glass flask;

– Distilled water;

– Disinfecting solution.

7. PREPARATION OF SPECIMENS

Use blood serum (plasma) specimens for the analysis.

Do not use strongly hemolyzed or turbid sera.

Blood serum (plasma) specimens can be stored at (2–8) °C for no longer than 5 days (provided there is no microbial contamination) or at minus 20 °C (and below) for no longer than 3 months. Avoid multiple freezing and thawing as this can lead to incorrect results. Thoroughly mix the specimens after thawing.

Blood serum (plasma) specimens containing precipitates should be centrifuged.

7.1. Preliminary dilution of the specimens

Dilute the specimens 10 times with SPSD. Pipette 90 µl of SPSD into the wells of the plate for pre-dilution of test samples and add 10 µl of blood serum (plasma) specimens, mix thoroughly. After dilution of serum, the color should change from red to yellow. If the color does not change, the analysis of serum specimens may give an incorrect result. When diluting plasma, the color of the solution in the well may change only slightly.

Store: for up to 3 hours at (18–26) °C.

8. PREPARATION OF KIT COMPONENTS

Prior to the analysis, open the kit package and keep all kit components at (18–26) °C for at least 30 min.

In the case of fractional use of the kit, after taking a portion of the solution immediately close the vials tightly with screw caps, put them into a refrigerator and store at (2–8) °C for the entire shelf life. PBS-T×25 and Stop solution can be stored at (2–30) °C.

8.1. Preparation of strips

Open the pouch above the zipper and fasten the number of strips required for the ELISA run in the frame of the plate. Use it within 1 hour.

Put the remaining strips immediately back into the foil pouch, squeeze the air out and tightly close the zipper.

Store: at (2–8) °C for the entire shelf life of the kit.

8.2. Preparation of Positive and Negative Control samples

Control samples (C^+, C^-) are ready for use and do not require additional dilution.

Store: at (2–8) °C for the entire shelf life of the kit.

8.3. Preparation of Wash solution

Shake the vial with PBS-T×25 concentrate before use. If salt precipitate is formed in the concentrate, heat it at (30–40) °C until complete dissolution of the precipitate.

Prepare Wash solution by diluting the provided PBS-T×25 concentrate 25 times. To do this, pipette the required volume of PBS-T×25 concentrate according to the number of used strips (see Table 1) into the measuring cylinder and add distilled water to the target volume. Mix thoroughly.

Store: Wash solution at (2–8) °C for no more than 1 month or at (18–26) °C for no more than 7 days.

Store: PBS-T×25 at (2–30) °C for the entire shelf life of the kit.

8.4. Preparation of Conjugate

Conjugate is ready for use. Pipette the required volume of Conjugate corresponding to the number of used strips (see Table 1) into a clean vial or plastic container for reagents.

Note: After working with Conjugate, wash the container with tap water and thoroughly rinse with distilled water. Never wash the container with detergents and disinfectants.

Store: at (2–8) °C for the entire shelf life of the kit.

8.5. Preparation of TMB solution

TMB solution is ready for use. Pipette the required volume of TMB solution corresponding to the number of used strips (see Table 1) into a clean vial or plastic container for reagents.

Avoid direct light on TMB solution.

Note: After working with TMB solution, rinse the container with water, wash with 70% ethanol, and thoroughly rinse with distilled water. Never wash the container with detergents and disinfectants. Even the traces of detergents may lead to incontrollable decomposition of TMB over the course of the reaction.

Store: at (2–8) °C for the entire shelf life of the kit.

Table 1

The required amount of reagents

Number of strips used	Conjugate, ml	TMB solution, ml	Wash solution	
			PBS-T×25, ml	Distilled water, ml
1	1.0	1.0	2.0	Up to 50
2	2.0	2.0	4.0	Up to 100
3	3.0	3.0	6.0	Up to 150
4	4.0	4.0	8.0	Up to 200
5	5.0	5.0	10.0	Up to 250
6	6.0	6.0	12.0	Up to 300
7	7.0	7.0	14.0	Up to 350
8	8.0	8.0	16.0	Up to 400
9	9.0	9.0	18.0	Up to 450
10	10.0	10.0	20.0	Up to 500
11	11.0	11.0	22.0	Up to 550
12	12.0	12.0	24.0	Up to 600

8.6. Preparation of Stop solution

Stop solution is ready for use.

Store: at (2–30) °C for the entire shelf life of the kit.

9. IMMUNOASSAY PROCEDURE

9.1. Sample addition

Shake Serum diluent (SD) before use.

Sample addition time should not exceed 10 min.

Add Control samples:

– **1 well** – 100 µl C^+;

– **2 wells** – 100 µl C^- each.

For example, add 100 µl of C^+ into A-1 well and 100 µl of C^- into B-1 and C-1 wells.

Add 90 µl of SD and 10 µl of pre-diluted specimens (see 7.1) into the remaining wells, mix by pipetting. For more reliable results, analyze specimens in doubles (use two wells for each specimen).

To determine antibody titer in positive samples, add 100 µl of C^+ into A-1 well and 100 µl of C^- into B-1 and C-1 wells. Pipette 180 µl of SD and 20 µl of pre-diluted specimens (see 7.1) into the wells of the horizontal row (A-2 – A-12). Mix by pipetting. Add 100 µl of SD into the remaining wells (B–H rows). Titrate each test sample (do not titrate C^- and C^+) by transferring 100 µl of the diluted samples from the wells of row A into the wells of row B and thoroughly mix them by pipetting. Continue the titration by transferring 100 µl of the diluted samples from the wells of the preceding row into the wells of the next row from B to H, thoroughly mixing them by pipetting each time. After titration, dispose of 100 µl of the well contents from each well of the last row H. Thus, consecutive 2-fold dilutions of the test samples are performed in the vertical rows of the plate (from 1:100 to 1:12800).

9.2. Incubation

Seal the plate strips with adhesive film and incubate in a thermostat for 30 min at 37 °C.

9.3. Washing

After the incubation, remove the adhesive film and put it into the container with a disinfecting solution. Wash the wells of the plate 5 times with Wash solution (see 8.3). Alternate an aspiration and immediate filling of the wells of each strip. Add at least 400 µl of Wash solution into each well during every washing cycle.

The time interval between the filling of the wells and aspiration should be not less than 30 seconds. Make sure that the wells are completely empty after

each washing cycle. After washing, remove the remaining liquid by tapping the upside-down plate on the filter paper.

9.4. Conjugate addition

Pipette 100 µl of Conjugate (see 8.4) into each well.

When pipetting Conjugate, use a plastic container and disposable pipette tips provided with the kit.

Note: Dispose of the remaining Conjugate from the vial or container (do not pour it back into the original vial with Conjugate).

9.5. Incubation

Seal the plate strips with adhesive film and incubate in a thermostat for 30 min at 37 °C.

9.6. Washing

After the incubation, wash the plate 5 times as described above.

9.7. TMB solution addition

Pipette 100 µl of TMB solution (see 8.5) into each well.

When pipetting TMB solution, use a plastic container and disposable pipette tips provided with the kit.

Note: Dispose of the remaining TMB solution from the vial or container (do not pour it back into the original vial with TMB solution).

9.8. Incubation

Incubate the strips in a dark place at (18–26) °C for 25 min.

9.9. Stop solution addition

Pipette 100 µl of Stop solution into each well at the same rate and in the same order as TMB solution.

A brief ELISA protocol is given in Annex 1 at the end of this Instruction Manual.

10. MEASUREMENT

Measure the optical density of Control (C^+, C^-) and test samples using a plate reader in two-wavelength mode: the main filter at 450 nm, the reference filter –

in the range of 620–655 nm. Measurement with a single filter at 450 nm is also allowed.

For the test samples with $OD_{sample\ 450} > 3.5$, repeat the measurement of OD in the mode: the main filter at 405 nm, the reference filter in the range 620–655 nm. Measurement with a single filter at 405 nm is also allowed.

Measurements must be carried out no later than 5 min after stopping the reaction.

11. DATA ANALYSIS AND INTERPRETATION

11.1. Calculate the average optical density in the wells with C^- samples ($OD_{av}C^-$).

11.2. Validation of the diagnostic test

The results of the analysis are considered valid only if the following conditions are met:

- The optical density in the well with C^+ sample is above or equal to 0.80;
- The average optical density in the wells with C^- samples is less than or equal to 0.25.

11.3. Interpretation of the results using a cutoff value

Calculate the cutoff value of the optical density using the following formula:

$$\mathbf{OD_{co} = OD_{av}C^- + 0.3,}$$

where $OD_{av}C^-$ is the average value of the optical density in the wells with Negative Control sample.

The result of the test is considered **positive** if $OD_{sample} \geq OD_{co}$, where OD_{sample} is the optical density of the test sample (or the average optical density in the pair of wells).

The result of the test is considered **negative** if $OD_{sample} \leq 0.85 \times OD_{co}$.

The result of the test is considered **equivocal** if $0.85 \times OD_{co} < OD_{sample} < OD_{co}$. In this case, it is recommended to analyze the specimen collected from the same patient after 2–4 weeks. If the OD is again in the range of $0.85 \times OD_{co}$–OD_{co} the result is considered negative.

11.4. Determination of antibody titer in positive samples

The results of the test are evaluated as described above.

The titer is considered the last dilution of the test sample, at which $OD_{sample} \geq OD_{co}$.

11.5. Interpretation of the results using a positivity coefficient

The results can be assessed using a positivity coefficient (PC).

To calculate the positivity coefficient of samples with $OD_{sample\ 450} \leq 3.5$, use the following formula:

$$PC_{sample} = \frac{OD_{sample\ 450}}{OD_{co}},$$

where $OD_{sample\ 450}$ is the optical density of the test sample obtained in two-wavelength mode 450 nm/620–655 nm (or with a single filter at 450 nm).

To calculate the positivity coefficient of samples with $OD_{sample\ 450} > 3.5$, use the following formula:

$$PC_{sample} = 3.2 \times \frac{OD_{sample\ 405}}{OD_{co}},$$

where $OD_{sample\ 405}$ is the optical density of the test sample obtained in two-wavelength mode 405 nm/620–655 nm (or with a single filter at 405 nm).

The result of the test is considered **positive** if $PC_{sample} \geq 1$, where PC_{sample} is the positivity coefficient of the test sample.

The result is considered **negative** if $PC_{sample} \leq 0.85$.

The result is considered **equivocal** if $0.85 < PC_{sample} < 1$.

Calculation of PC is useful for assessing the concentration of IgG to *Clonorchis* antigens in test samples and monitoring of the concentration changes in paired serum (plasma) samples.

12. STORAGE AND TRANSPORTATION

– Transport the kit at (2–8) °C. Transportation at the temperature up to 26 °C for no more than 10 days is acceptable.

– Store the kit in the manufacturer's packaging at (2–8) °C for the entire shelf life.

– The shelf life of the kit is 12 months from the manufacture date.

13. WARRANTY

The manufacturer hereby guarantees the conformity of manufactured products to the requirements of normative and technical documentation.

Safety and quality of products are guaranteed throughout the entire shelf life.

The manufacturer is responsible for product's unsatisfactory features, except for the defects that have arisen as a result of a violation of the Instruction Manual, transportation and storage conditions, actions of third parties or force majeure.

The manufacturer shall replace the product at its own expense if technical and functional characteristics of the product do not comply with the normative and technical documentation and these disadvantages are caused by a latent defect in material or defective manufacturing.

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BRIEF ELISA PROTOCOL

“Clonorchis-IgG-EIA-BEST” kit

Use only after the careful study of the Instruction Manual!

Add:	100 µl of C^+ and C^-;
	90 µl of SD and 10 µl of pre-diluted specimens.
Incubate:	30 min, 37 °C.
Wash:	Wash solution, 400 µl, 5 times.
Add:	100 µl of Conjugate.
Incubate:	30 min, 37 °C.
Wash:	Wash solution, 400 µl, 5 times.
Add:	100 µl of TMB solution.
Incubate:	25 min, (18–26) °C, in a dark place.
Add:	100 µl of Stop solution.
Measure:	OD at 450 nm/reference wavelength 620–655 nm.

EXPLANATION OF SYMBOLS

REF	Catalog number	IVD	*In vitro* diagnostic medical device
Σ n	Contains sufficient for <n> tests		Temperature limit
LOT	Lot number		Manufacturer
	Date of manufacture: XXXX-XX-XX Date format: Year-Month-Day		Consult Instruction Manual
	Use before: XXXX-XX-XX Date format: Year-Month-Day		Caution! Consult Instruction Manual