Vitrotest Helicobacter pylori IgA

ELISA test kit for the qualitative and semiquantitative determination of IgA class antibodies to *Helicobacter pylori*

REF EL099-96

IVD Σ 96

1. INTENDED USE

The test kit Vitrotest Helicobacter pylori IgA is an enzyme linked immunosorbent assay (ELISA) for the qualitative and semiquantitative determination of IgA antibodies to *Helicobacter pylori* in human serum or plasma.

The test kit might be applied for the ELISA using both automatic pipettes and standard equipment as well as open system automated ELISA analyzers.

2. CLINICAL VALUE

Helicobacter pylori is a widespread microorganism infecting half of the world's population. Its prevalence is very high in developing countries and is quite low in the developed world. According to the World Gastroenterology Organization, infection of adults in Eastern Europe and Asia is 70-80%.

Studies during recent decades have shown the key role of bacterium *Helicobacter pylori* in the pathogenesis of stomach and duodenal lesions. *H. pylori* is detected almost in 100 % of adult patients with duodenal ulcer, approximately in 80 % of patients with peptic ulcer, in 92 % of patients with gastric cancer and in 92 % of patients with active chronic gastritis. Research has demonstrated that elimination of helicobacter leads to the disappearance of gastritis and significant reduction in the incidence of duodenal ulcer recurrence.

Helicobacteriosis is a chronic infection with long, often asymptomatic course. Its symptoms do not differ from clinical manifestations of gastro-duodenitis since usual constant pain in the epigastrium occurs. *H. pylori* is often present in patients with no clinical manifestations of disease.

H. pylori strains are extremely heterogeneous and divided into two groups: strains that express both antigens VacA and CagA (type I) and strains that do not express these antigens (type II). Strains of the first group dominate in patients with peptic ulcer disease and gastric cancer. CagA protein penetrates the epithelial cells of the mucous membrane, causes abnormal mitosis and induces chromosome instability. Organism infected with expressing CagA *H. pylori* produces antibodies, which are specific for CagA. Antibodies to CagA protein are found in 80-100 % of patients with duodenal ulcer and in 94 % of patients with gastric cancer. Thus, detection of the antibodies to CagA protein is an informative marker in the diagnosis of duodenal ulcer and gastric cancer.

Strains of *H. pylori* II type which do not express antigens CagA and VacA are associated with severe gastric and duodenal ulcers, particularly peptic ulcer disease and cancer.

H. pylori infection of the patient can be identified with both invasive and non-invasive diagnostic methods. Invasive methods include bioptic studies of the gastrointestinal tract mucosa with histological or culture methods or a rapid urease test. However, heterogeneous spread of *H. pylori* in tissues often leads to false-negative results. Non-invasive diagnostic methods include serologic studies of patient sera for the presence of specific antibodies to *H. pylori* and respiratory urease test using radioactively labeled urea. ELISA for the detection of specific IgG/IgA/IgM antibodies is the least invasive, rapid, highly sensitive and informative method for the diagnosis of *H. pylori* infection.

3. PRINCIPLE OF THE TEST

Determination of IgA antibodies to *H. pylori* in the test kit Vitrotest Helicobacter pylori IgA is based on a solid phase, indirect ELISA in a two-step incubation procedure. Microwells are coated a mixture of recombinant and native *H. pylori* antigens. During the first incubation step, the specific antibodies to *H. pylori*, if present in the sample, will be bound to the solid phase precoated antigens. The wells are washed to remove unbound antibodies. A secondary antibody (anti-IgA), which is conjugated to horseradish peroxidase (HRP), is added next and binds to the immune complexes on the solid phase. Unbound components are removed by washing. Antigen-antibody complexes are revealed by addition of chromogen solution containing 3,3',5,5'- tetramethylbenzidine (TMB) and hydrogen peroxide. After 15 minutes the reaction has been stopped, the absorbance values are read using a spectrophotometer at 450/620-695 nm. The colour intensity is proportional to the amount of the antibodies present in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Composition of the test kit

ELISA STRIPS	1x96 wells	Microplate (12 strips x 8 wells) Each well is coated with a mixture of recombinant and native *H. pylori* antigens. The wells can be separated.
CONTROL +	1x0.5 ml	Positive control Solution of specific monoclonal immunoglobulins with preservative (pink)

CONTROL –	1x0.5 ml	**Negative control** Buffer solution with detergent and preservative (yellow).
CONTROL CUT-OFF	1x0.5 ml	**Cut-off control** Solution of specific monoclonal immunoglobulins with preservative (orange).
SAMPLE DILUENT	1x12 ml	**Sample diluent** Buffer solution with detergent and preservative (brown-green).
CONJUGATE SOLUTION	1x12 ml	**Conjugate solution** Buffer solution of monoclonal antibodies to human IgA conjugated to HRP with stabilizers and preservative (orange), ready to use.
TMB SOLUTION	1x12 ml	**TMB solution** TMB, H_2O_2, stabilizers, preservative (colourless), ready to use.
WASH TWEEN 20X	1x50 ml	**Washing solution Tw20 (20x concentrate)** 20X concentrated of PBS buffer with Tween-20 and NaCl (colourless)
STOP SOLUTION	1x12 ml	**Stop Solution** 0.5 mol/l H_2SO_4 (colourless), ready to use.

Adhesive films (2), sera identification plan (1), instruction for use and certificate of analysis.

4.2. Material required but not provided

- variable volume automatic pipettes (10µl–1000 µl) and disposable pipette tips;
- plate reader (single wavelength 450 nm or dual wavelength 450/620–695 nm);
- volumetric laboratory glassware (10–1000 ml);
- distilled/DI water;
- incubator thermostatically controlled at 37 °C;
- automatic/semiautomatic plate washer;
- appropriate waste containers for potentially contaminated materials;
- timer;
- absorbent paper;
- disposable gloves;
- disinfectants;
- protective clothes.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

The ELISA assays are time and temperature sensitive. Strictly follow the test procedure and do not modify it.

- do not use expired reagents;
- do not use for analyses and do not mix reagents from different lots or from test kits of different nosology as well as other manufacturer's reagents with Vitrotest kits;

Note: it is possible to use WASH TWEEN 20X, TMB SOLUTION *and* STOP SOLUTION *from other Vitrotest ELISA kits.*

- close reagents after use only with appropriate caps;
- control the filling and full aspiration of the solution in the wells;
- use a new tip for each sample and reagent;
- avoid exposure of kit reagents to direct sunlight;
- TMB SOLUTION must be colourless before use. If TMB SOLUTION is blue or yellow it cannot be used. Avoid any contact of TMB SOLUTION with metals or metal ions. Use glassware thoroughly washed and rinsed with distilled/DI water;
- never use the same glassware for CONJUGATE SOLUTION and TMB SOLUTION.

The manufacturer is not responsible or liable for any incorrect results and/or incidents taking place as a result of any violation of the instruction. The manufacturer is not responsible for visual readings of samples (without using a plate reader).

5.2. Safety

- all components of test kit are intendent for *in vitro* diagnostic use only;
- all materials of human or animal origin should be regarded and handled as potentially infectious;
- the ELISA is only designed for qualified personnel;

– disposable gloves and safety glasses must be worn at all times while performing analysis;
– never eat, drink, smoke or apply cosmetics in the assay laboratory;
– never pipette solutions by mouth;
– controls do not contain of human origin components;
– avoid contact with STOP SOLUTION containing 0.5 mol/l H_2SO_4. It may cause skin irritation and burns;
– some components of the test kit contain low concentrations of harmful compounds and could cause irritation of the skin and the mucosa. In the case of contact of TMB SOLUTION, STOP SOLUTION or CONJUGATE SOLUTION with skin or mucosa, the place of contact should be immediately rinsed with large amounts of water;
– in case of spilling of solutions that do not contain acid, e.g. sera, rinse the surface with disinfectant, then dry it with absorbent paper. In other case acid first must be neutralized by sodium bicarbonate and then wiped out as described above;
– for information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request.

5.3. Waste treatment

Patient specimens, controls, and incubated microplate strips should be treated as infectious waste, residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

6. STORAGE AND STABILITY

Reagents are stable until stated expiration date on the label when stored refrigerated (2-8°C). Do not freeze. The kit should be shipped at 2-8°C. Single transportation at the temperature up to 23°C for two days is possible.

After the first opening of the packaging, the components of the ELISA kits are stable within 3 months, except for those specified in p. 8 of this Instruction.

7. SPECIMEN COLLECTION

The fresh serum or plasma (EDTA, lithium-heparin, sodium citrate, potassium fluoride) samples can be stored for 3 days at 2-8 °C, or frozen for longer periods at -20 – -70 °C. Frozen samples must be thawed and kept at room temperature for at least 30 min before use. Do not use preheated samples. Mix thawed samples thoroughly to homogeneity. Avoid repeated freezing/thawing. Samples containing aggregates must be clarified by centrifugation (3000 rpm for 10-15 min). Do not use hyperlipeamic, hyperhaemolysed or contaminated by microorganisms serum specimens. The presence of bilirubin up to concentration of 0.21 mg/ml (361.8 µmol/l), haemoglobin up to concentration of 10 mg/ml and triglycerides up to concentration of 10 mg/ml (11.3 mmol/l) are allowed.

8. REAGENT PREPARATION

It is very important to keep all test components for at least 30 min at room temperature (18-25 ºC) before the assay!

8.1. ELISA STRIPS preparation

Before opening the bag with the ELISA STRIPS, keep it at room temperature for 30 minutes to avoid water condensation inside the wells. Open the vacuum bag and take out the necessary number of wells. Once opened, the bag with the remaining strips and desiccant must be ***resealed with the zip-lock*** immediately and kept refrigerated at 2-8°C for no more than 3 months.

8.2. Washing solution preparation

Check the WASH TWEEN 20X for the presence of salt crystals. If crystals have formed, re-solubilise them by warming the vial at 37°C, until crystals have been fully dissolved (15-20 min). Dilute the WASH TWEEN 20X 1:20 (1+19) with distilled/DI water before use. For example, 4ml concentrate + 76 ml water is sufficient for 8 wells. Once diluted it is stable at 2-8°C for 7 days.

9. ASSAY PROCEDURE

9.1 Take out from the protective bag the support frame and the necessary number of the wells ELISA STRIPS (the number of specimens + 4 for controls). Place the wells into the frame. Wells with the controls must be included in every test.
9.2 Complete the sera identification plan.
9.3 Prepare washing solution (see 8.2.).
9.4 Dispense 90 µl of SAMPLE DILUENT into each well.
9.5. Dispense 10 µl of controls and patient samples into the wells in the following order: A1 – CONTROL +, B1, C1 – CONTROL CUT-OFF, and D1 – CONTROL –, other wells – patient samples. Mix gently to avoid foaming. The colour of the sample diluent changes from brown -green to blue.
9.6 Cover strips with an adhesive film and incubate for 30 min at 37°C.

9.7 Remove and discard the adhesive film and wash all wells 5 times with automatic washer or 8-channel pipette as follows:
- aspirate the contents of all wells into a liquid waste container and add immediately a minimum of 300 µl of diluted Washing solution to each well;
- soak each well for 30 seconds between each wash cycle;
- aspirate again. The residual volume must be lower than 5µl.
- repeat the washing step 4 times;
- after the final washing cycle, turn down the plate onto an absorbent paper and tap it to remove any residual buffer.

9.8 Dispense 100 µl of CONJUGATE SOLUTION per well. Cover strips with a new adhesive film, incubate for 30 min at 37°C.
9.9 Remove and discard the adhesive film and wash all wells five times as described above (see 9.7).
9.10 Dispense 100 µl TMB SOLUTION into all wells. Do not touch the walls and bottoms of the wells to avoid contamination.
9.11 Incubate the strips for 15 minutes at room temperature (18-25°C) in the dark. Do not use adhesive film in this step.
9.12 Add 100 µl STOP SOLUTION to each well in the same order and at the same rate as for TMB SOLUTION.
9.13 Read the optical density (OD) of the wells at 450/620-695 nm using a microplate reader within 5 minutes after adding the STOP SOLUTION. Pay attention to the cleanness of the plate bottom and absence of bubbles in the wells before reading.

Measurement in the single-wave procedure at 450 nm is possible. Reserve blank well to adjust spectrophotometer in such analysis. Only TMB SOLUTION *and* STOP SOLUTION *must be added in blank well).*

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Validation of the test

The test run may be considered valid provided the following criteria are met:

CONTROL +	OD ≥ 1.2
CONTROL CUT-OFF	OD in a range 0.25-0.65
CONTROL –	OD ≤ 0.150

If one of the control cut-off absorbances does not match the above criteria, this value should be discarded and a mean value should be calculated using the remaining cut-off value. If both control cut-off absorbance do not meet the criteria, the test is invalid and must be re-tested.

10.2. Calculation of results

The cut-off (CO) is the mean optical density (OD) of the wells containing CONTROL CUT-OFF:

$$CO = (OD_{CONTROL\ CUT\text{-}OFF\ 1} + OD_{CONTROL\ CUT\text{-}OFF\ 2})/2;$$

The sample result is reported as a Ratio:

$$Ratio_{sample} = OD_{sample}/CO, \quad OD_{sample} - \text{optical density of the well containing sample}$$

10.3. Interpretation of results

$Ratio_{sample} > 1.1$	POSITIVE
$0.9 \leq Ratio_{sample} \leq 1.1$	DOUBTFUL*
$Ratio_{sample} < 0.9$	NEGATIVE

* If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

11. PERFORMANCE CHARACTERISTICS

11.1. Specificity and sensitivity

Sensitivity of the test kit Vitrotest Helicobacter pylori IgA was evaluated using 20 sera which contain IgG antibodies to *H. pylori*. In the test kit Vitrotest Helicobacter pylori IgA among IgG positive sera 17 samples were identified as positive. The data obtained were confirmed by an CE-marked enzyme immunoassay for the detection of IgA antibodies against *H. pylori*. In the test kit Vitrotest Helicobacter pylori IgA sensitivity was 85%.

In the study of 23 negative for antibodies to *H. pylori* sera, specificity rate of the test kit Vitrotest Helicobacte pylori IgA was above 91.3%.

11.2. Accuracy

Intra assay repeatability

Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 32-replicate determinations using 1 lot of the test kit.

Serum No.	OD	Ratio	CV, %
H3	0.917	2.94	7.5
H4	0.526	1.69	5.1

Inter assay reproducibility

Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 4 ELISA performances during 4 days, in 8-replicate determinations.

Serum No.	OD	Ratio	CV, %
H3	0.851	2.73	7.4
H4	0.529	1.70	5.7

12. LIMITATIONS OF THE PROCEDURE

A positive result in the test kit Vitrotest Helicobacter pylori IgA indicates the presence of specific antibodies IgA to *H. pylori*.

A negative result in the test kit Vitrotest Helicobacter pylori IgA indicates the absence in serum of IgA antibodies specific to *H. pylori*.

For a comprehensive serological diagnosis of *H. pylori*-infection it is recommended to test the samples for the presence IgG specific antibodies to *H. pylori* using, for example, test kit Vitrotest Helicobacter pylori IgG.

It should be noted that in case of early *H. pylori* infection the result may be negative due to the absence or low concentration of antibodies. In the presence of clinical manifestations of the disease it is recommended to repeat the test at least in two weeks.

Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis, in fact, should take into consideration as well as clinical history, symptomatology and serological data.

13. TROUBLESHOOTING

Possible causes	*Solutions*
High background in all wells	
Contaminated washer	Clean the washer head, then rinse it with 30% ethanol and distilled water
Low quality water or contaminated water	Use distilled/DI with resistivity ≥ 10 MΩ·cm.
Using contaminated glassware	Use clean glassware
Using chlorine based disinfectants	Use disinfectants without chlorine
Using contaminated tips	Use new tips
Increased time of incubation or temperature regimen was changed	Follow incubation regimen according to instruction for use
High background in a few wells	
TMB solution was added more than once	Add TMB solution once
Pipette shaft was contaminated with conjugate solution	Clean the pipette; pipette the liquids carefully
One the channels of the washer was contaminated	Clean the washer channel, clean the washer
OD of the positive control below normal	
Conjugate solution/tmb solution was prepared improperly or not added	Run ELISA repeatedly, prepare conjugate solution / TMB solution properly

Reduced incubation time in one of the stages	Follow incubation regimen according to the instruction for use
Visual colour intensity of the wells does not correspond to optical density	
The optical beam or another component of the reader is misaligned or malfunctioning	Test the absorbance reader's performance

REFERENCE

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Catalogue number


Consult instructions for use

In vitro diagnostic medical device


Manufacturer


Caution


Contains sufficient for <*n*> tests


Temperature limit

Batch code


Use-by date


Date of manufacture


Keep away from sunlight


Signifies European conformity (CE) mark

Inst_ Helicobacter_pylori_IgA_EL099-96_V01_ENG
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Vitrotest Europe Sp. z o.o.
Krakowska str., 139-155, 50-428, Wroclaw, Poland
tel.: +48 882 950 379,
e-mail: info@vitrotest.pl, www.vitrotest.pl

Vitrotest Helicobacter pylori IgA

ASSAY PROCEDURE

Keep all reagents and specimens for at least 30 min at 18-25 °C before use

Dispense 90 µl of SAMPLE DILUENT into the wells
(brown-green colour)

Dispense 10 µl of controls and samples into the wells in the following order:
A1 – CONTROL +,
B1, C1 – CONTROL CUT-OFF,
D1 – CONTROL –,
E1 and other wells – patient samples
(colour changes from brown-green to blue)

Cover wells with an adhesive film, incubate for 30 min at 37 °C

Rinse the wells 5 times with diluted 1:20 (1+19) washing solution Tween-20 (300 µl per well)

Add 100µl of CONJUGATE SOLUTION into the wells
(orange colour)

Cover wells with an adhesive film, incubate for 30 min at 37 °C

Rinse the wells 5 times with diluted 1:20 (1+19) washing solution Tween-20 (300 µl per well)

Add 100 µl of TMB SOLUTION into the wells

Incubate for 15 min in the dark at 18-25 °C

Add 100 µl of STOP SOLUTION
(colour changes from blue to yellow)

Determine the optical density (OD) at 450/620-695 nm

CALCULATION

$CO = (OD_{CONTROL\ CUT\text{-}OFF\ 1} + OD_{CONTROL\ CUT\text{-}OFF\ 2})/2;$

$Ratio_{sample} = OD_{sample}/CO$

INTERPRETATION

$Ratio_{sample} > 1.1$	POSITIVE
$0.9 \leq Ratio_{sample} \leq 1.1$	DOUBTFUL
$Ratio_{sample} < 0.9$	NEGATIVE

Vitrotest SARS-CoV-2 IgM

ELISA test kit for the qualitative determination of IgM class antibodies to coronavirus SARS-CoV-2

REF EL034-96

Σ 96

IVD

1. INTENDED USE

The test kit Vitrotest SARS-CoV-2 IgM is an enzyme linked immunosorbent assay (ELISA) for the qualitative determination of IgM class antibodies to N (nucleocapsid) and S (spike) antigens of SARS-CoV-2 coronavirus in human serum or plasma.

The test kit might be applied for the ELISA using both automatic pipettes and standard equipment as well as open system automated ELISA analyzers.

2. CLINICAL VALUE

COVID-19 is an infectious disease caused by a new SARS-CoV-2 coronavirus which had not previously been detected in humans.

The viral infection leads to the development of a respiratory flu-like disease with symptoms such as cough and fever. In more severe cases pneumonia can develop. The average incubation period of the COVID-19 is 6.5 days, but it can range from 3 to 21 days.

Coronavirus SARS-CoV-2 is an RNA-containing virus with a characteristic envelope with spikes in the form of a “corona”. The main structural proteins of the virus include the nucleocapsid protein and transmembrane S (spike) protein with a receptor-binding domain (RBD), which binds to human cell receptors ACE2, causing infection of the mucosal epithelial cells. Both proteins are highly immunogenic antigens for humans.

Specific antibodies (IgM, IgG, IgA) to the SARS-CoV-2 N- and S-proteins appear on the 7-11th day from the moment the virus enters / contacts the body of an infected person. Anti-SARS-CoV-2 IgM and IgA antibodies can be detected as early as 4 days after the first symptoms of the disease. N- and S-specific IgM in not requiring intensive care patients may peak in the second week after COVID-19 symptoms appear. According to the literature, the simultaneous determination of N- and S-specific IgM antibodies makes it possible to identify up to 75 % of patients infected with SARS-CoV-2 in the first week of clinical manifestations.

At the same time, the level of specific IgG continues to grow and can be detected in almost 100 % of patients (except for those with immunosuppression) by the 20th day from the onset of COVID-19 symptoms.

3. PRINCIPLE OF THE TEST

Determination of IgM antibodies specific to SARS-CoV-2 in the test kit Vitrotest SARS-CoV-2 IgM is based on “IgM-capture” solid phase ELISA in a two-step incubation procedure. Microwells are coated with monoclonal antibodies to human IgM. During the first incubation step, IgM antibodies will be bound to the solid phase precoated monoclonal antibodies. The wells are washed to remove unbound components. Recombinant antigens of SARS-CoV-2 (N- and S- antigens), which is conjugated to horseradish peroxidase (HRP), is added next and binds to the specific IgM captured on the solid phase. Unbound components are removed by washing. Antigen-antibody complexes are revealed by addition of chromogen solution containing 3,3',5,5'- tetramethylbenzidine (TMB) and hydrogen peroxide. After 30 minutes the reaction has been stopped, the absorbance values are read using a spectrophotometer at 450/620-695 nm. The colour intensity is proportional to the amount of the specific antibodies present in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Composition of the test kit

ELISA STRIPS	1x96 wells	Microplate (12 strips x 8 wells) Each well is coated with monoclonal antibodies to human IgM. The wells can be separated.
CONTROL +	1x0.5 ml	Positive control Solution of specific monoclonal immunoglobulins with preservative (pink).
CONTROL –	1x0.5 ml	Negative control Buffer solution with detergent and preservative (yellow).
CONTROL CUT-OFF	1x0.5 ml	Cut-off control Solution of specific monoclonal immunoglobulins with preservative (orange).

SAMPLE DILUENT	1x12 ml	**Sample diluent** Buffer solution with detergent and preservative (violet).
CONJUGATE SOLUTION	1x12 ml	**Conjugate solution** Buffer solution of recombinant antigens of SARS-CoV-2 conjugated to HRP with stabilizers and preservative (green), ready to use.
TMB SOLUTION	1x12 ml	**TMB solution** TMB, H_2O_2, stabilizers, preservative (colourless), ready to use.
WASH TWEEN 20X	1x50 ml	**Washing solution Tw20 (20x concentrate)** 20X concentrated of PBS buffer with Tween-20 and NaCl (colourless)
STOP SOLUTION	1x12 ml	**Stop Solution** 0,5 mol/l H_2SO_4 (colourless), ready to use.

Adhesive films (2), sera identification plan (1), instruction for use and certificate of analysis.

4.2. Material required but not provided

- variable volume automatic pipettes (10µl–1000 µl) and disposable pipette tips;
- plate reader (single wavelength 450 nm or dual wavelength 450/620–695 nm);
- volumetric laboratory glassware (10–1000 ml);
- distilled/DI water;
- incubator thermostatically controlled at 37 °C;
- automatic/semiautomatic plate washer;
- appropriate waste containers for potentially contaminated materials;
- timer;
- absorbent paper;
- disposable gloves;
- disinfectants;
- protective clothes.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

The ELISA assays are time and temperature sensitive. Strictly follow the test procedure and do not modify it.

- do not use expired reagents;
- do not use for analyses and do not mix reagents from different lots or from test kits of different nosology as well as other manufacturer's reagents with Vitrotest kits;

Note: it is possible to use WASH TWEEN 20X, TMB SOLUTION *and* STOP SOLUTION *from other Vitrotest ELISA kits.*

- close reagents after use only with appropriate caps;
- control the filling and full aspiration of the solution in the wells;
- use a new tip for each sample and reagent;
- avoid exposure of kit reagents to direct sunlight;
- TMB SOLUTION must be colourless before use. If TMB SOLUTION is blue or yellow it cannot be used. Avoid any contact of TMB SOLUTION with metals or metal ions. Use glassware thoroughly washed and rinsed with distilled/DI water;
- never use the same glassware for CONJUGATE SOLUTION and TMB SOLUTION.

The manufacturer is not responsible or liable for any incorrect results and/or incidents taking place as a result of any violation of the instruction. The manufacturer is not responsible for visual readings of samples (without using a plate reader).

5.2. Safety

- all components of test kit are intendent for *in vitro* diagnostic use only;
- all materials of human or animal origin should be regarded and handled as potentially infectious;
- the ELISA is only designed for qualified personnel;
- disposable gloves and safety glasses must be worn at all times while performing analysis;
- never eat, drink, smoke or apply cosmetics in the assay laboratory;
- never pipette solutions by mouth;
- controls do not contain of human origin components;
- avoid contact with STOP SOLUTION containing 0.5 mol/l H_2SO_4. It may cause skin irritation and burns;
- some components of the test kit contain low concentrations of harmful compounds and could cause irritation of the skin and the mucosa. In the case of contact of TMB SOLUTION, STOP SOLUTION or CONJUGATE SOLUTION with skin or mucosa, the place of contact should be immediately rinsed with large amounts of water;

– in case of spilling of solutions that do not contain acid, e.g. sera, rinse the surface with disinfectant, then dry it with absorbent paper. In other case acid first must be neutralized by sodium bicarbonate and then wiped out as described above;
– for information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request.

5.3. Waste treatment

Patient specimens, controls, and incubated microplate strips should be treated as infectious waste, residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

6. STORAGE AND STABILITY

Reagents are stable until stated expiration date on the label when stored refrigerated (2-8 °C). Do not freeze. The kit should be shipped at 2-8 °C. Single transportation at the temperature up to 23 °C for two days is acceptable.

After the first opening of the packaging, the components of the ELISA kits are stable within 3 months, except for those specified in p. 8 of this Instruction.

7. SPECIMEN COLLECTION

The fresh serum or plasma (EDTA, lithium-heparin, sodium citrate) samples can be stored for 3 days at 2-8 °C, or frozen for longer periods at -20 – -70 °C. Frozen samples must be thawed and kept at room temperature for at least 30 min before use. Do not use preheated samples. Mix thawed samples thoroughly to homogeneity. Avoid repeated freezing/thawing. Samples containing aggregates must be clarified by centrifugation (3000 rpm for 10-15 min). Do not use hyperlipeamic, hyperhaemolysed or contaminated by microorganisms serum specimens. The presence of bilirubin up to concentration of 0.21 mg/ml (361.8 µmol/l), haemoglobin up to concentration of 10 mg/ml and triglycerides up to concentration of 10 mg/ml (11.3 mmol/l) are allowed.

8. REAGENT PREPARATION

It is very important to keep all test components for at least 30 min at room temperature (18-25 °C) before the assay!

8.1. ELISA STRIPS preparation

Before opening the bag with ELISA STRIPS, keep it at room temperature for 30 min to avoid water condensation inside the wells. Open the vacuum bag and take out the necessary number of the wells. Once opened the bag with the remaining strips must be ***resealed with zip-lock*** immediately and kept refrigerated at 2-8 °C for no more than 3 months.

8.2. Washing solution preparation

Check the WASH TWEEN 20X for the presence of salt crystals. If crystals have formed, re-solubilise by warming at 37°C, until crystals dissolve (15-20 min). Dilute the WASH TWEEN 20X 1:20 (1+19) with distilled/DI water before use. For example, 4 ml concentrate + 76 ml water is sufficient for 8 wells. Once diluted it is stable at 2-8 °C for 7 days.

9. ASSAY PROCEDURE

9.1. Take out from the protective bag the support frame and the necessary number of the wells ELISA STRIPS (the number of specimens + 4 for controls). Place the wells into the frame. Wells with the controls must be included in every test.
9.2. Complete the sera identification plan.
9.3. Prepare washing solution (see 8.2.).
9.4. Dispense 90 µl of SAMPLE DILUENT into each well ELISA STRIPS.
9.5. Dispense 10 µl of controls and patient samples into the wells in the following order: A1 – CONTROL +, B1, C1 – CONTROL CUT-OFF, and D1 – CONTROL –, other wells – patient samples. Mix gently to avoid foaming. The colour of the sample diluent changes from violet to blue.
9.6. Cover strips with an adhesive film and incubate for 30 min at 37 °C.
9.7. Remove and discard the adhesive film and wash all wells 5 times with automatic washer or 8-channel pipette as follows:
– aspirate the contents of all wells into a liquid waste container and add immediately a minimum of 300 µl of diluted washing solution to each well;
– soak each well for 30 s between each wash cycle;
– aspirate again. The residual volume must be lower than 5 µl;
– repeat the washing step 4 times;
– after the final washing cycle, turn down the plate onto an absorbent paper and tap it to remove any residual buffer.

9.8. Dispense 100 µl of CONJUGATE SOLUTION per well. Cover strips with a new adhesive film, incubate for 30 min at 37 °C.
9.9. Remove and discard the adhesive film and wash all wells five times as described above (see 9.7).
9.10. Dispense 100 µl TMB SOLUTION into all wells. Do not touch the walls and bottoms of the wells to avoid contamination.
9.11. Incubate the strips for 30 min at room temperature (18-25 °C) in the dark. Do not use adhesive film in this step.
9.12. Add 100 µl STOP SOLUTION to each well in the same order and at the same rate as for TMB SOLUTION.
9.13. Read the optical density (OD) of the wells at 450/620-695 nm using a microplate reader within 5 min after adding the STOP SOLUTION. Pay attention to the cleanness of the plate bottom and absence of bubbles in the wells before reading.

Measurement in the single-wave procedure at 450 nm is possible. Reserve blank well to adjust spectrophotometer in such analysis. Only TMB SOLUTION *and* STOP SOLUTION *must be added in blank well.*

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Validation of the test

The test run may be considered valid provided the following criteria are met:

CONTROL +	OD ≥ 1.2
CONTROL CUT-OFF	OD in a range 0.25-0.65
CONTROL –	OD ≤ 0.150

If one of the control cut-off absorbances does not match the above criteria, this value should be discarded and a mean value should be calculated using the remaining cut-off value. If both control cut-off absorbance do not meet the criteria, the test is invalid and must be re-tested.

10.2. Calculation of results

The cut-off (CO) is the mean optical density (OD) of the wells containing CONTROL CUT-OFF:

$$CO = (OD_{\text{CONTROL CUT-OFF 1}} + OD_{\text{CONTROL CUT-OFF 2}})/2;$$

The sample result is reported as a Ratio:

$$Ratio_{sample} = OD_{sample}/CO, \quad OD_{sample} – \text{optical density of the well containing sample}$$

10.3. Interpretation of results

$Ratio_{sample} > 1.1$	POSITIVE
$0.9 \le Ratio_{sample} \le 1.1$	DOUBTFUL*
$Ratio_{sample} < 0.9$	NEGATIVE

* If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

11. PERFORMANCE CHARACTERISTICS

11.1. Specificity and sensitivity

To assess the diagnostic characteristics of the Vitrotest SARS-CoV-2 IgM test kit we used Anti-SARSCoV-2 Verification Panel for Serology Assays (manufactured by NIBSC, UK), which contains 23 blood plasma samples from COVID-19 convalescents and 14 negative blood plasma samples. The results obtained in the Vitrotest SARS-CoV-2 IgM test kit completely coincide with the panel passport data.

In a comparative studies of the sensitivity of the Vitrotest SARS-CoV-2 IgM test kit on 85 samples, which were determined as positive in a commercial analogue recommended by the FDA, the percentage of agreement of the obtained results was 96.5 %.

To determine the sensitivity of the Vitrotest SARS-CoV-2 IgM test kit in the early stages of SARS-CoV-2 infection, 1117 blood sera obtained 5-15 days from the onset of clinical manifestations of COVID-19 were tested. Specific IgM antibodies were detected in 972 samples.

The specificity of the Vitrotest SARS-CoV-2 IgM test kit on 706 human blood serum samples obtained during the first half of 2019 (before the start of the COVID-19 pandemic) was 99.4 %.

11.2. Accuracy

Intra assay repeatability

Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 32-replicate determinations using 1 lot of the test kit.

Serum No.	OD	Ratio	CV, %
336	0.755	3.45	2.7
345	2.408	11.02	1.6

Inter assay reproducibility

Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 4 ELISA performances during 4 days, in 8-replicate determinations.

Serum No.	OD	Ratio	CV, %
336	0.758	3.46	2.4
345	2.409	10.99	1.2

12. LIMITATIONS OF THE PROCEDURE

If the test sample was obtained in the first days after infection IgM antibodies may not be detected. Therefore, a negative result of the test for IgM antibodies to SARS-CoV-2 does not exclude the infection of the patient with the virus. In the presence of clinical manifestations of the disease it is recommended to repeat the test at least in 1-2 weeks, for example when using test kits Vitrotest SARS-CoV-2 IgM, Vitrotest SARS-CoV-2 IgG and Vitrotest SARS-CoV-2 Total Ab.

Negative test results in immunosuppressed individuals should also be interpreted with caution.

The final diagnosis cannot be established solely on the basis of serological test results. The diagnosis should take into consideration clinical history, symptomatology, as well as the results of other laboratory tests (including PCR).

13. TROUBLESHOOTING

Possible causes	*Solutions*
High background in all wells	
Contaminated washer	Clean the washer head, then rinse it with 30 % ethanol and distilled water
Low quality water or contaminated water	Use distilled/DI with resistivity ≥ 10 MΩ·cm.
Using contaminated glassware	Use clean glassware
Using chlorine based disinfectants	Use disinfectants without chlorine
Using contaminated tips	Use new tips
Increased time of incubation or temperature regimen was changed	Follow incubation regimen according to instruction for use
High background in a few wells	
TMB solution was added more than once	Add TMB solution once
Pipette shaft was contaminated with conjugate solution	Clean the pipette; pipette the liquids carefully
One the channels of the washer was contaminated	Clean the washer channel, clean the washer
OD of the positive control below normal	
Conjugate solution/tmb solution was prepared improperly or not added	Run ELISA repeatedly, prepared conjugate solution / TMB solution properly
Reduced incubation time in one of the stages	Follow incubation regimen according to the instruction for use
Visual colour intensity of the wells does not correspond to optical density	
The optical beam or another component of the reader is misaligned or malfunctioning	Test the absorbance reader's performance

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Catalogue number


Consult instructions for use

In vitro diagnostic medical device


Manufacturer


Caution


Contains sufficient for <*n*> tests


Temperature limit

Batch code


Use-by date


Date of manufacture


Keep away from sunlight


Signifies European conformity (CE) mark

Inst_SARS-CoV-2_IgM_EL034-96_V01_ENG
Edition 1st, 16.12.2021

Vitrotest Europe Sp. z o.o.
Krakowska str., 139-155, 50-428, Wroclaw, Poland
tel.: +48 882 950 379,
e-mail: info@vitrotest.pl, www.vitrotest.pl

Vitrotest SARS-CoV-2 IgM

ASSAY PROCEDURE

Keep all reagents and specimens for at least 30 min at 18-25 °C before use

Dispense 90 µl of SAMPLE DILUENT into the wells of ELISA STRIPS.
(violet colour)

Dispense 10 µl of controls and samples into the wells in the following order:
A1 – CONTROL +,
B1, C1 – CONTROL CUT-OFF,
D1 – CONTROL –,
E1 and other wells – patient samples
(colour changes from violet to blue)

Cover wells with an adhesive film, incubate for 30 min at 37 °C

Rinse the wells 5 times with diluted 1:20 (1+19) washing solution Tween-20 (300 µl per well)

Add 100 µl of CONJUGATE SOLUTION into the wells
(green colour)

Cover wells with an adhesive film, incubate for 30 min at 37 °C

Rinse the wells 5 times with diluted 1:20 (1+19) washing solution Tween-20 (300 µl per well)

Add 100 µl of TMB SOLUTION into the wells

Incubate for 30 min in the dark at 18-25 °C

Add 100 µl of STOP SOLUTION
(colour changes from blue to yellow)

Determine the optical density (OD) at 450/620-695nm

CALCULATION

$CO = (OD_{CONTROL\ CUT\text{-}OFF\ 1} + OD_{CONTROL\ CUT\text{-}OFF\ 2})/2;$

$Ratio_{sample} = OD_{sample}/CO$

INTERPRETATION

$Ratio_{sample} > 1.1$	POSITIVE
$0.9 \leq Ratio_{sample} \leq 1.1$	DOUBTFUL
$Ratio_{sample} < 0.9$	NEGATIVE

Vitrotest SARS-CoV-2 IgG QuantiSpike

ELISA test kit for the quantitative determination of IgG class antibodies to coronavirus SARS-CoV-2 Spike protein

REF EL040-96

IVD Σ 96

1. INTENDED USE

The test kit Vitrotest SARS-CoV-2 IgG QuantiSpike is an enzyme linked immunosorbent assay (ELISA) for the quantitative determination of IgG class antibodies to SARS-CoV-2 Spike protein synthesized in humans due to the disease or vaccination in serum or plasma.

The test kit might be applied for the ELISA using both automatic pipettes and standard equipment as well as open system automated ELISA analyzers.

2. CLINICAL VALUE

COVID-19 is an infectious disease caused by a new SARS-CoV-2 coronavirus which had not previously been detected in humans.

The viral infection leads to the development of a respiratory flu-like disease with symptoms such as cough and fever. In more severe cases pneumonia can develop. The average incubation period of the COVID-19 is 6.5 days, but it can range from 3 to 21 days.

SARS-CoV-2 is an RNA-virus with a specific envelope with spikes in the form of a “corona”. The main structural proteins of the virus include envelope protein (E), membrane protein (M), spike (S) glycoprotein, and nucleocapsid (N) protein. S protein on the surface of the SARS-CoV-2 virion mediates the receptor recognition and cell membrane fusion with ACE2 molecules, which are mainly expressed on type II pneumocytes, colon and kidney epithelial cells. It contains three fragments, namely the ectodomain, the transmembrane domain and the short intracellular segment. The ectodomain consists of a receptor-binding subunit S1 containing the RBD domain and a fusion subunit (S2). During viral infection, S1 C-terminal domain binds to the extracellular peptidase (PD) domain of ACE2 to ensure that the virus attaches to the surface of the target cell. The S1 N-terminal domain binds to glycans causing the cleavage of S protein between S1 and S2 fragments by cellular proteases, which, in turn, initiates the fusion of viral and cell membranes by the S2 subunit.

Although most viral proteins are able to induce the production of specific antibodies after SARS-CoV-2 infection, and antibodies to N- and S-protein are widely used in the serological diagnosis of COVID-19, antibodies targeting viral S-protein are more noteworthy because they can block SARS-CoV-2 entry into the host cells. And since most vaccines induce antibodies to the spike protein the determination of IgG specific to this antigen also makes it possible to assess the presence of protective antibodies after the disease or vaccination against COVID-19.

3. PRINCIPLE OF THE TEST

Determination of IgG antibodies to S-protein of SARS-CoV-2 in the test kit Vitrotest SARS-CoV-2 IgG QuantiSpike is based on a solid phase, indirect ELISA in a two-step incubation procedure. Microwells are coated with the recombinant antigen, SARS-CoV-2 S-protein analogue. During the first incubation step, the specific antibodies to SARS-CoV-2 S-protein, if present in the sample, will be bound to the solid phase precoated antigens. The wells are washed to remove unbound antibodies. A secondary antibody (anti-IgG), which is conjugated to horseradish peroxidase (HRP), is added next and binds to the immune complexes on the solid phase. Unbound components are removed by washing. Antigen-antibody complexes are revealed by addition of chromogen solution containing 3,3’,5,5’-tetramethylbenzidine (TMB) and hydrogen peroxide. After 15 min the reaction has been stopped, the absorbance values are read using a spectrophotometer at 450/620-695 nm. The colour intensity is proportional to the amount of the antibodies present in the sample.

Internal calibrators of the Vitrotest SARS-CoV-2 IgG QuantiSpike test kit are standardized according to the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (human) code: 20/136 (NIBSC, UK), which contains 1000 binding antibody units (BAU) per ml.

4. MATERIALS AND EQUIPMENT

4.1. Composition of the test kit

ELISA STRIPS	1x96 wells	Microplate (12 strips x 8 wells) Each well is coated with the recombinant antigens, SARS-CoV-2 S-protein analogues. The wells can be separated.

PREDILUTION PLATE	1x96 wells	Microplate for preliminary dilution of sera
CAL 0	1x0.3 ml	Calibrator 0 Buffer solution with detergent and preservative (yellow).
CAL 25	1x0.3 ml	Calibrator 25 Solution of specific monoclonal immunoglobulins to S-protein (25 BAU/ml) with stabilizers and preservative (green).
CAL 50	1x0.3 ml	Calibrator 50 Solution of specific monoclonal immunoglobulins to S-protein (50 BAU/ml) with stabilizers and preservative (orange).
CAL 100	1x0.3 ml	Calibrator 100 Solution of specific monoclonal immunoglobulins to S-protein (100 BAU/ml) with stabilizers and preservative (pink).
CAL 200	1x0.3 ml	Calibrator 200 Solution of specific monoclonal immunoglobulins to S-protein (200 BAU/ml) with stabilizers and preservative (violet).
CONTROL +	1x0.5 ml	Positive control Solution of specific monoclonal immunoglobulins with a known concentration to S-protein with stabilizers and preservative (red).
SAMPLE PREDILUENT	1x50 ml	Sample prediluent Buffer solution with detergent and preservative (brown-green).
SAMPLE DILUENT	1x12 ml	Sample diluent Buffer solution with detergent and preservative (yellow).
CONJUGATE SOLUTION	1x12 ml	Conjugate solution Buffer solution of monoclonal antibodies to human IgG conjugated to HRP with stabilizers and preservative (violet), ready to use.
TMB SOLUTION	1x12 ml	TMB solution TMB, H_2O_2, stabilizers, preservative (colourless), ready to use.
WASH TWEEN 20X	1x50 ml	Washing solution Tw20 (20x concentrate) 20X concentrated of phosphate buffer with Tween-20 and NaCl (colourless).
STOP SOLUTION	1x12 ml	Stop Solution 0.5 mol/l H_2SO_4 (colourless), ready to use.

Adhesive films (2), sera identification plan (1), blank calibration curve (1), instruction for use and certificate of analysis.

4.2. Material required but not provided

- Variable volume automatic pipettes (10µl–1000 µl) and disposable pipette tips;
- plate reader (single wavelength 450 nm or dual wavelength 450/620–695 nm);
- volumetric laboratory glassware (10–1000 ml);
- distilled/DI water;
- incubator thermostatically controlled at 37 °C;
- automatic/semiautomatic plate washer;
- appropriate waste containers for potentially contaminated materials;
- timer;
- absorbent paper;
- disposable gloves;
- disinfectants;
- protective clothes.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

The ELISA assays are time and temperature sensitive. Strictly follow the test procedure and do not modify it.

- do not use expired reagents;
- do not use for analyses and do not mix reagents from different lots or from test kits of different nosology as well as other manufacturer's reagents with Vitrotest kits;

Note: it is possible to use WASH TWEEN 20X, TMB SOLUTION, STOP SOLUTION *and* SAMPLE PREDILUENT *from other lots.*

- close reagents after use only with appropriate caps;
- control the filling and full aspiration of the solution in the wells;
- use a new tip for each sample and reagent;
- avoid exposure of kit reagents to direct sunlight;
- TMB SOLUTION must be colourless before use. If TMB SOLUTION is blue or yellow it cannot be used. Avoid any contact of TMB SOLUTION with metals or metal ions. Use glassware thoroughly washed and rinsed with distilled/DI water;
- never use the same glassware for CONJUGATE SOLUTION and TMB SOLUTION.

The manufacturer is not responsible or liable for any incorrect results and/or incidents taking place as a result of any violation of the instruction. The manufacturer is not responsible for visual readings of samples (without using a plate reader).

5.2. Safety

- all components of test kit are intendent for *in vitro* diagnostic use only;
- all materials of human or animal origin should be regarded and handled as potentially infectious;
- the ELISA is only designed for qualified personnel;
- disposable gloves and safety glasses must be worn at all times while performing analysis;
- never eat, drink, smoke or apply cosmetics in the assay laboratory;
- never pipette solutions by mouth;
- controls do not contain of human origin components;
- avoid contact with STOP SOLUTION containing 0.5 mol/l H_2SO_4. It may cause skin irritation and burns;
- some components of the test kit contain low concentrations of harmful compounds and could cause irritation of the skin and the mucosa. In the case of contact of TMB SOLUTION, STOP SOLUTION or CONJUGATE SOLUTION with skin or mucosa, the place of contact should be immediately rinsed with large amounts of water;
- in case of spilling of solutions that do not contain acid, e.g. sera, rinse the surface with disinfectant, then dry it with absorbent paper. In other case acid first must be neutralized by sodium bicarbonate and then wiped out as described above;
- for information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request.

5.3. Waste treatment

Patient specimens, calibrators, control and incubated microplate strips should be treated as infectious waste, residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

6. STORAGE AND STABILITY

Reagents are stable until stated expiration date on the label when stored refrigerated (2-8 °C). Do not freeze. The kit should be shipped at 2-8 °C. Single transportation at the temperature up to 23 °C for two days is acceptable.

After the first opening of the packaging, the components of the ELISA kits are stable within 3 months, except for those specified in p. 8 of this Instruction.

7. SPECIMEN COLLECTION

The fresh serum or plasma (EDTA, lithium-heparin) samples can be stored for 3 days at 2-8 ºC, or frozen for longer periods at -20 – -70 °C. Frozen samples must be thawed and kept at room temperature for at least 30 minutes before use. Do not use preheated samples. Mix thawed samples thoroughly to homogeneity. Avoid repeated freezing/thawing. Samples containing aggregates must

be clarified by centrifugation (3000 rpm for 10-15 min). Do not use hyperlipeamic, hyperhaemolysed or contaminated by microorganisms serum specimens. The presence of bilirubin up to concentration of 0.21 mg/ml (361.8 µmol/l), haemoglobin up to concentration of 10 mg/ml and triglycerides up to concentration of 10 mg/ml (11.3 mmol/l) are allowed.

8. REAGENT PREPARATION

It is very important to keep all test components for at least 30 min at room temperature (18-25 ºC) before the assay!

8.1. ELISA STRIPS preparation

Before opening the bag with the ELISA STRIPS, keep it at room temperature for 30 min to avoid water condensation inside the wells. Open the vacuum bag and take out the necessary number of wells. Once opened, the bag with the remaining strips and desiccant must be ***resealed with the zip-lock*** immediately and kept refrigerated at 2-8 °C for no more than 3 months.

8.2. Washing solution preparation

Check the WASH TWEEN 20X for the presence of salt crystals. If crystals have formed, re-solubilise them by warming the vial at 37 °C, until crystals have been fully dissolved (15-20 min). Dilute the WASH TWEEN 20X 1:20 (1+19) with distilled/DI water before use. For example, 4ml concentrate + 76 ml water is sufficient for 8 wells. Once diluted it is stable at 2-8 °C for 7 days.

8.3. Predilution of samples, calibrators and positive control

Predilute patient samples, calibrators and positive control 1:10 with SAMPLE PREDILUENT immediately before test. Dispense 90 µl of SAMPLE PREDILUENT in the wells of PREDILUTION PLATE, add 10 µl of samples, calibrators and positive control. Gently mix the content in the wells. After addition of the sample colour of the sample prediluent changes from brown-green to blue.

The procedure for dilution of samples, controls and calibrators should be carried out immediately before analysis.

9. ASSAY PROCEDURE

9.1. Take out from the protective bag the support frame and the necessary number of the wells ELISA STRIPS (the number of specimens, 1 well for the positive control and 5 wells for calibrators). Place the wells into the frame. Wells with calibrators and positive control must be included in every test.
9.2. Complete the sera identification plan.
9.3. Prepare washing solution (see 8.2.).
9.4. Predilute patient samples, calibrators and positive control (see 8.3).
9.5. Dispense 90 µl of SAMPLE DILUENT in the wells of ELISA STRIPS.
9.6. Add 10 µl of prediluted (1:10) calibrators, positive control and patient samples to the wells in the following order: A1– CAL 200, B1 - CAL 100, C1 - CAL 50, D1 - CAL 25, E1 - CAL 0 and F1 – CONTROL + respectively; other wells – patient samples. The final dilution in the wells is 1:100. Pipette gently to avoid foaming. The colour of the sample diluent changes from yellow to green.

Given the technical features of the equipment used for analysis, the order of dispense calibrators can be reversed: A1 - CAL 0, B1 - CAL 25, C1 - CAL 50, D1 - CAL 100, E1 - CAL 200.

9.7. Cover strips with an adhesive film and incubate for 30 min at 37 °C.
9.8. Remove and discard the adhesive film and wash all wells 5 times with automatic washer or 8-channel pipette as follows:
- aspirate the contents of all wells into a liquid waste container and add immediately a minimum of 300 µl of diluted washing solution to each well;
- soak each well for 30 s between each wash cycle;
- aspirate again. The residual volume must be lower than 5 µl;
- repeat the washing step 4 times;
- after the final washing cycle, turn down the plate onto an absorbent paper and tap it to remove any residual buffer.

9.9. Dispense 100 µl CONJUGATE SOLUTION per well. Cover strips with a new adhesive film, incubate for 30 min at 37 °C.
9.10. Remove and discard the adhesive film and wash all wells five times as described above (see 9.8).
9.11 Dispense 100 µl TMB SOLUTION into all wells. Do not touch the walls and bottoms of the wells to avoid contamination.

9.12. Incubate the strips for 15 min at room temperature (18-25 °C) in the dark. Do not use adhesive film in this step.
9.13 Add 100 µ STOP SOLUTION to each well in the same order and at the same rate as for TMB SOLUTION.
9.14. Read the optical density (OD) of the wells at 450/620-695 nm using a microplate reader within 5 min after adding the STOP SOLUTION. Pay attention to the cleanness of the plate bottom and absence of bubbles in the wells before reading.

Measurement in the single-wave procedure at 450 nm is possible. Reserve blank well to adjust spectrophotometer in such analysis. Only TMB SOLUTION *and* STOP SOLUTION *must be added in blank well.*

10. CALCULATION AND INTERPRETATION OF RESULTS

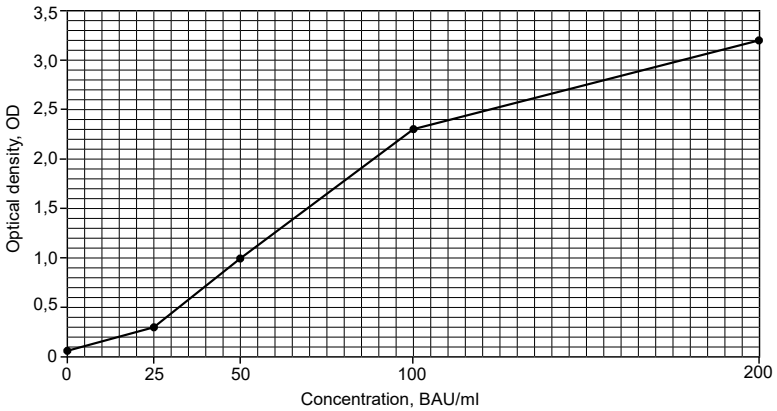
10.1. Validation of the test

The test run may be considered valid provided the following criteria are met:

CAL 0	OD ≤ 0.100
CAL 25	OD ≥ 0.120
CAL 200	OD ≥ 1.500
CONTROL +	Within the concentration range on the tube label and on the certificate of analysis

10.2. Calculation of results

To determine the concentration of specific IgG antibodies in BAU/ml build a calibration curve. Plot the absorbances (OD) for 5 calibrators CAL 0, CAL 25, CAL 50, CAL 100 and CAL 200 on Y axis and their corresponding concentrations in BAU/ml (0, 25, 50, 100 and 200 BAU/ml) respectively, on X axis. Using the absorbance values for each sample and positive control determine the corresponding concentration (BAU/ml) from the calibration curve.



Note that the calibration curve is intended for use only as an example, not for calculation of your results.

In case of OD of patient samples is higher of CAL 200 the test result will be ">200 BAU/ml". Such samples can be retested at a dilution of 1:800. For diluted samples (1:800) multiply calculated results by 8.

final concentration = concentration by the calibration curve × 8

If the optical density of the samples at a dilution of 1:800 is still above the value CAL 200 it is recommended to retest such samples at a dilution of 1:1000 and 1:4000. In this case, the concentration of specific antibodies should be multiplied by the dilution rate of 10 and 40, respectively.

It is recommended to use computer software to read and calculate the obtained results.

10.3 Interpretation of results

Ig G concentration	Interpretation
> 25 BAU/ml	POSITIVE
20 - 25 BAU/ml	DOUBTFUL*
< 20 BAU/ml	NEGATIVE

* If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

11. PERFORMANCE CHARACTERISTICS

11.1. Specificity and sensitivity

The specificity of the test kit Vitrotest SARS-CoV-2 IgG QuantiSpike using 352 samples of human sera obtained in the first half of 2019 (before the COVID-19 pandemic) was 100 %.

The sensitivity of the test kit Vitrotest SARS-CoV-2 IgG QuantiSpike, using 49 serum samples of COVID-19 convalescents (obtained 2-10 months after the disease), was 100 %. In addition, when testing 18 serum samples of vaccinated individuals all samples contained IgG antibodies to spike protein at a concentration of more than 1000 BAU/ml.

Diagnostic characteristics of the test kit Vitrotest SARS-CoV-2 IgG QuantiSpike were also evaluated on the verification panel Anti-SARSCoV-2 Verification Panel for Serology Assays code: 20/B770 (manufactured by NIBSC, UK), which consists of 23 characterized plasma samples of COVID-19 convalescents containing antibodies to SARS-CoV-2, and 14 characterized blood plasma samples not containing antibodies to SARS-CoV-2. The sensitivity and specificity of the test kit on this panel was 100%.

In the study of samples of the First WHO International Reference Panel for anti-SARS-CoV-2 immunoglobulin code: 20/268 (manufactured by NIBSC, UK) in the test kit Vitrotest SARS-CoV-2 IgG QuantiSpike obtained results matched the panel passport data.

11.2. Accuracy

Intra assay repeatability

Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 32-replicate determinations using 1 lot of the test kit.

Serum No.	OD	Concentration, BAU/ml	CV, %
783	0.671	35.9	5.5
977	2.222	92.3	7.5

Inter assay reproducibility

Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 4 ELISA performances during 4 days, in 8-replicate determinations.

Serum No.	OD	Concentration, BAU/ml	CV, %
783	0.643	34.8	5.3
977	2.143	93.4	6.9

11.3 Analytical sensitivity

The limit of determination (LOD), the lowest concentration of the analyte in the sample, which is detected with the declared probability for the test kit Vitrotest SARS-CoV-2 IgG QuantiSpike is 3.5 BAU/ml.

11.4. Linearity range

The linearity range of the test kit Vitrotest SARS-CoV-2 IgG QuantiSpike is within 10-164 BAU/ml.

11.5. Compliance of the test-kit calibrators with the International Standard

Vitrotest SARS-CoV-2 IgG QuantiSpike calibrators comply with the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (human) code: 20/136 (NIBSC, UK). The coefficient of determination (R2) is 0.99.

12. LIMITATIONS OF THE PROCEDURE

If the test sample was obtained in the first days after the infection IgG antibodies may not be detected. Therefore a negative result does not exclude the SARS-CoV-2 infection. In the presence of clinical manifestations of the disease it is recommended to repeat the test in 1-2 weeks.

Negative test results in immunosuppressed individuals should also be interpreted with caution.

The diagnosis should take into consideration clinical history, symptomatology, as well as the results of other laboratory tests.

13. TROUBLESHOOTING

Possible causes	*Solutions*
	High background in all wells
Contaminated washer	Clean the washer head, then rinse it with 30 % ethanol and distilled water
Low quality water or contaminated water	Use distilled/DI with resistivity ≥ 10 MΩ·cm.
Using contaminated glassware	Use clean glassware
Using chlorine based disinfectants	Use disinfectants without chlorine
Using contaminated tips	Use new tips
Increased time of incubation or temperature regimen was changed	Follow incubation regimen according to instruction for use
	High background in a few wells
TMB solution was added more than once	Add TMB solution once
Pipette shaft was contaminated with conjugate solution	Clean the pipette; pipette the liquids carefully
One the channels of the washer was contaminated	Clean the washer channel, clean the washer
	OD of the positive control below normal
Conjugate solution/tmb solution was prepared improperly or not added	Run ELISA repeatedly, prepared conjugate solution / TMB solution properly
Reduced incubation time in one of the stages	Follow incubation regimen according to the instruction for use
	Visual colour intensity of the wells does not correspond to optical density
The optical beam or another component of the reader is misaligned or malfunctioning	Test the absorbance reader's performance

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Catalogue number


Consult instructions for use

In vitro diagnostic medical device


Manufacturer


Caution


Contains sufficient for <*n*> tests


Temperature limit

Batch code


Use-by date


Date of manufacture


Keep away from sunlight


Signifies European conformity (CE) mark

Inst_SARS-CoV-2-IgG_QuantiSpike_IgG_EL040-96_V01_ENG
Edition 1st, 16.12.2021

Vitrotest Europe Sp. z o.o.
Krakowska str., 139-155, 50-428, Wroclaw, Poland
tel.: +48 882 950 379,
e-mail: info@vitrotest.pl, www.vitrotest.pl

CE

Vitrotest SARS-CoV-2 IgG QuantiSpike

ASSAY PROCEDURE

Keep all reagents and specimens for at least 30 min at 18-25 °C before use

Dispense 90 µl of SAMPLE PREDILUENT (brown-green colour) into the wells of PREDILUTION PLATE and add 10 µl of calibrators, positive control and the samples
(colour changes from brown-green to blue)

Dispense 90 µl of SAMPLE DILUENT *(yellow colour)* into the wells of ELISA STRIPS and add 10 µl of prediluted calibrators, positive control and samples into the wells of ELISA STRIPS in the following order:
A1 - CAL 200, B1 - CAL 100, C1 - CAL 50, D1 - CAL 25, E1 - CAL 0 *(or vice versa: A1 - CAL 0, B1 - CAL 25, C1 - CAL 50, D1 - CAL 100, E1 - CAL 200)*, F1 - CONTROL +, G1 and other wells – patient samples
(colour changes from yellow to green)

Cover wells with an adhesive film, incubate for 30 min at 37 °C

Rinse the wells 5 times with diluted 1:20 (1+19) washing solution Tween-20 (300 µl per well)

Add 100 µl of CONJUGATE SOLUTION to each well
(violet colour)

Cover wells with an adhesive film, incubate for 30 min at 37 °C

Rinse the wells 5 times with diluted 1:20 (1+19) washing solution Tween-20 (300 µl per well)

Add 100 µl of TMB SOLUTION to each well

Incubate the plate for 15 min in the dark at 18-25 °C

Stop the reaction by adding 100 µl of STOP SOLUTION
(colour changes from blue to yellow)

Determine the optical density (OD) at 450/620-695 nm

Build a calibration curve, determine the concentration of IgG specific antibodies to SARS-CoV-2 S-protein in the samples and interpret the test results according to the table:

IgG concentration	Interpretation
> 25 BAU/ml	POSITIVE
20-25 BAU/ml	DOUBTFUL
< 20 BAU/ml	NEGATIVE

INSTRUCTION FOR USE

Vitrotest EBV EBNA-1 IgG

ELISA test kit for the qualitative and semiquantitative determination of IgG class antibodies to nuclear antigen of Epstein-Barr Virus

REF EL054-96

IVD Σ 96

1. INTENDED USE

The test kit Vitrotest EBV EBNA-1 IgG is an enzyme linked immunosorbent assay (ELISA) for the qualitative and semiquantitative determination of IgG class antibodies to nuclear antigen (EBNA) of Epstein-Barr virus (EBV) in human serum or plasma.

The test kit might be applied for the ELISA using both automatic pipettes and standard equipment as well as open system automated ELISA analyzers.

2. CLINICAL VALUE

Epstein-Barr virus (EBV), also known as human herpesvirus 4, is the causative agent of diseases such as infectious mononucleosis (IM), Burkitt's lymphoma (BL), nasopharyngeal carcinoma.

EBV acquired in early childhood usually does not cause symptoms. Infection in adolescence or early adulthood often results in clinical IM. Typically, the disease presents as pharyngitis, lymphadenopathy, and fever. In most cases, symptoms resolve within 2–4 weeks, but more than 90% of adults develop a latent B-lymphocyte infection. Approximately 1% of immunocompetent individuals may experience severe complications (hepatitis, myocarditis, splenic rupture, neurological complications). In immunosuppressed individuals, primary EBV infection leads to severe disorders (eg, BL).

For laboratory diagnosis of EBV infection, a polymerase chain reaction and serological methods are used that include a test for the detection of heterophilic antibodies and the detection of specific antibodies by ELISA. The latter method allows not only to establish the fact of infection with EBV, but also the stage of the disease.

The optimal combination of serological tests for the diagnosis of EBV infection involves the detection of IgG and IgM antibodies that are specific for the viral capsid antigen (anti-VCA-IgG and anti-VCA-IgM) and nuclear antigen (anti-EBNA-IgG). Anti-VCA-IgM antibodies appear in the body during an early infection and disappear within 4-12 weeks. IgG to VCA appear later, their concentration reaches a maximum level at the initial stages of the disease, gradually decreasing. However, these antibodies are determined throughout life. If antibodies to the viral capsid antigen do not appear, a person is susceptible to EBV infection.

Anti-EBNA-IgG are produced by the body 2-4 months after the onset of symptoms. The concentration of the antibodies is maintained at a high level for a long time or throughout life in the majority of infected people. Simultaneous detection of specific IgG to EBNA and VCA indicates the past infection.

3. PRINCIPLE OF THE TEST

Determination of IgG antibodies to EBV nuclear antigen (EBNA) in the test kit Vitrotest EBV EBNA-1 IgG is based on a solid phase, indirect ELISA in a two-step incubation procedure. Microwells are coated with EBV recombinant nuclear antigen. During the first incubation step, the specific antibodies to EBNA, if present, will be bound to the solid phase precoated antigens. The wells are washed to remove unbound antibodies, leaving only the specific antigen-antibody complexes. A secondary antibody (anti-IgG) which are conjugated to horseradish peroxidase (HRP) added next and bind to the immune complexes on the solid phase. Unbound components are removed by washing. Immune complexes are revealed by adding of chromogen solution containing 3,3',5,5'- tetramethylbenzidine (TMB) and hydrogen peroxide. After 15 minutes the reaction has been stopped, the absorbance values are read using a spectrophotometer at 450/620-695 nm. The colour intensity is proportional to the amount of antibody in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Composition of the test kit

ELISA STRIPS	1x96 wells	Microplate ELISA (12 strips x 8 wells) Each well is coated with EBV recombinant nuclear antigen. The wells can be separated.
CONTROL +	1x0.5 ml	Positive control Solution of specific monoclonal immunoglobulins with preservative (pink).
CONTROL –	1x0.5 ml	Negative control Buffer solution with detergent and preservative (yellow).

CONTROL CUT-OFF	1x0.5 ml	Cut-off control Solution of specific monoclonal immunoglobulins with preservative (orange).
SAMPLE DILUENT	1x12 ml	Sample diluent Buffer solution with detergent and preservative (brown-green).
CONJUGATE SOLUTION	1x12 ml	Conjugate solution Buffer solution of monoclonal antibodies to human IgG conjugated to HRP with stabilizers and preservative (green), ready to use.
TMB SOLUTION	1x12 ml	TMB solution TMB, H_2O_2, stabilizers, preservative (colourless), ready to use.
WASH TWEEN 20X	1x50 ml	Washing solution Tw20 (20x concentrate) 20X concentrated of PBS buffer with Tween-20 and NaCl (colourless).
STOP SOLUTION	1x12 ml	Stop solution 0.5 mol/l H_2SO_4 (colourless), ready to use.

Adhesive films (2), sera identification plan (1), instruction for use and certificate of analysis.

4.2. Material required but not provided

- variable volume automatic pipettes (10 µl–1000 µl) and disposable pipette tips;
- plate reader (single wavelength 450 nm or dual wavelength 450/620–695 nm);
- volumetric laboratory glassware (10–1000 ml);
- distilled or deionized water;
- incubator thermostatically controlled at 37 °C;
- automatic/semiautomatic plate washer;
- appropriate waste containers for potentially contaminated materials;
- timer;
- absorbent paper;
- disposable gloves;
- disinfectants;
- protective clothes.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

The ELISA assays are time and temperature sensitive. Strictly follow the test procedure and do not modify it.

- do not use expired reagents;
- do not use for analyses and do not mix reagents from different lots or from test kits of different nosology as well as other manufacturer's reagents with Vitrotest kits;

Note: it is possible to use WASH TWEEN 20X, TMB SOLUTION *and* STOP SOLUTION *from other Vitrotest ELISA kits.*

- close reagents after use only with appropriate caps;
- control the filling and full aspiration of the solution in the wells;
- use a new tip for each sample and reagent;
- avoid exposure of kit reagents to direct sunlight;
- TMB SOLUTION must be colourless before use. If TMB SOLUTION is blue or yellow it cannot be used. Avoid any contact of TMB SOLUTION with metals or metal ions. Use glassware thoroughly washed and rinsed with distilled/DI water;
- never use the same glassware for CONJUGATE SOLUTION and TMB SOLUTION.

The manufacturer is not responsible or liable for any incorrect results and/or incidents taking place as a result of any violation of the instruction. The manufacturer is not responsible for visual readings of samples (without using a plate reader).

5.2. Safety

- all components of test kit are intendent for *in vitro* diagnostic use only;
- all materials of human or animal origin should be regarded and handled as potentially infectious;
- the ELISA is only designed for qualified personnel;

– disposable gloves and safety glasses must be worn at all times while performing analysis;
– never eat, drink, smoke or apply cosmetics in the assay laboratory;
– never pipette solutions by mouth;
– controls do not contain of human origin components;
– avoid contact with STOP SOLUTION containing 0.5 mol/l H_2SO_4. It may cause skin irritation and burns;
– some components of the test kit contain low concentrations of harmful compounds and could cause irritation of the skin and the mucosa. In the case of contact of TMB SOLUTION, STOP SOLUTION or CONJUGATE SOLUTION with skin or mucosa, the place of contact should be immediately rinsed with large amounts of water;
– in case of spilling of solutions that do not contain acid, e.g. sera, rinse the surface with disinfectant, then dry it with absorbent paper. In other case acid first must be neutralized by sodium bicarbonate and then wiped out as described above;
– for information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request.

5.3. Waste treatment

Patient specimens, controls, and incubated microplate strips should be treated as infectious waste, residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

6. STORAGE AND STABILITY

Reagents are stable until stated expiration date on the label when stored refrigerated (2-8 °C). Do not freeze. The kit should be shipped at 2-8 °C. Single transportation at the temperature up to 23 °C for two days is acceptable.

After the first opening of the packaging, the components of the ELISA kits are stable within 3 months, except for those specified in p. 8 of this Instruction.

7. SPECIMEN COLLECTION

The fresh serum or plasma (EDTA, lithium-heparin, sodium citrate, potassium fluoride) samples can be stored for 3 days at 2-8 °C or frozen for longer periods at -20 – -70 °C. Frozen samples must be thawed and kept at room temperature for at least 30 min before use. Do not use preheated samples. Mix thawed samples thoroughly to homogeneity. Avoid repeated freezing/thawing. Samples containing aggregates must be clarified by centrifugation (3000 rpm for 10-15 min). Do not use hyperlipeamic, hyperhaemolysed or contaminated by microorganisms serum specimens. The presence of bilirubin up to concentration of 0.21 mg/ml (361.8 µmol/l), haemoglobin up to concentration of 10 mg/ml and triglycerides up to concentration of 10 mg/ml (11.3 mmol/l) are allowed.

8. REAGENT PREPARATION

It is very important to keep all test components for at least 30 min at room temperature (18-25 ºC) before the assay!

8.1. ELISA STRIPS preparation

ELISA STRIPS are vacuum packed with a desiccant to absorb moisture.

Before opening the bag with ELISA STRIPS, keep it at room temperature for 30 min to avoid water condensation inside the wells. Open the vacuum bag and take out the necessary number of the wells. Once opened the bag with the remaining strips and desiccant must be ***resealed with zip-lock*** immediately and kept refrigerated at 2-8 °C for no more than 3 months.

8.2. Washing solution preparation

Check the WASH TWEEN 20X for the presence of salt crystals. If crystals have formed, re-solubilise by warming at 37 °C, until crystals have been fully dissolved (15-20 min).

ilute the WASH TWEEN 20X 1:20 (1+19) with distilled or deionized water before use and mix. For example, 4 ml concentrate + 76 ml water is sufficient for 8 wells. Once diluted it is stable at 2-8 °C for 7 days.

9. ASSAY PROCEDURE

9.1. Take out from the protective bag the support frame and the necessary number of the wells ELISA STRIPS (the number of specimens + 4 for controls). Place the wells into the frame. Wells with the controls must be included in every test.
9.2. Complete the sera identification plan.
9.3. Prepare washing solution (see 8.2.).
9.4. Dispense 90 µl of SAMPLE DILUENT into each well.
9.5. Dispense 10 µl of controls and patient samples into the wells in the following order: A1 – CONTROL + , B1, C1 – CONTROL CUT-OFF, and D1 – CONTROL – , other wells – patient samples. Mix gently to avoid foaming. The colour of the sample diluent changes from brown -green to blue.

9.6. Cover strips with an adhesive film and incubate for 30 min at 37 °C.
9.7. At the end of the incubation period, remove and discard the adhesive film and wash the well 5 times with automatic washer or 8-channel pipette as follows:
- aspirate the contents of all wells into a liquid waste container and add immediately a minimum of 300 µl of diluted washing solution to each well;
- soak each well for 30 s between each wash cycle;
- aspirate again. The residual volume must be lower than 5 µl;
- repeat the washing step 4 times;
- after the final washing cycle, turn down the plate onto an absorbent paper and tap it to remove any residual buffer.

9.8. Dispense 100 µl of CONJUGATE SOLUTION per well. Cover strips with a new adhesive film, incubate for 30 min at 37°C.
9.9. At the end of the incubation period, remove and discard the adhesive film and wash the wells five times as described above (see 9.7).
9.10. Dispense 100 µl TMB SOLUTION into all wells. Do not touch the walls and bottoms of the wells to avoid contamination.
9.11. Incubate the strips for 15 min at room temperature (18-25 °C) in the dark. Do not use adhesive film in this step.
9.12. Dispense 100 µl STOP SOLUTION into all wells in the same order and at the same rate as for TMB SOLUTION.
9.13. Read the optical density (OD) of the wells at 450/620-695 nm using a microplate reader within 5 min after adding the STOP SOLUTION. Pay attention to the cleanness of the plate bottom and absence of bubbles in the wells before reading.

Measurement in the single-wave procedure at 450 nm is possible. Reserve blank well to adjust spectrophotometer in such analysis. Only TMB SOLUTION *and* STOP SOLUTION *must be added in blank well.*

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Validation of the test

The test run may be considered valid provided the following criteria are met:

CONTROL +	OD ≥ 1.2
CONTROL CUT-OFF	OD in a range 0.25-0.70
CONTROL –	OD ≤ 0.150

If one of the control cut-off absorbances does not match the above criteria, this value should be discarded and a cut-off value should be calculated using the remaining cut-off control. If both control cut-off absorbance do not meet the criteria, the test is invalid and must be repeated.

10.2. Calculation of results

The cut-off (CO) is the mean optical density (OD) of the wells containing CONTROL CUT-OFF:

$$CO = (OD_{CONTROL\ CUT\text{-}OFF\ 1} + OD_{CONTROL\ CUT\text{-}OFF\ 2})/2.$$

The sample result is reported as a Ratio:

$$Ratio_{sample} = OD_{sample}/CO,$$

OD_{sample} – optical density of the well containing sample

10.3. Interpretation of results

$Ratio_{sample} > 1.0$	POSITIVE
$0.8 \leq Ratio_{sample} \leq 1.0$	DOUBTFUL*
$Ratio_{sample} < 0.8$	NEGATIVE

* If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

11. PERFORMANCE CHARACTERISTICS

11.1. Specificity and sensitivity

In the comparative studies of the test kit Vitrotest EBV EBNA-1 IgG with other CE marked ELISA kit 136 serum samples that contained IgG antibodies to EBV nuclear antigen and 124 serum samples that did not contain serological markers of the EBV infection. Relative sensitivity and specificity of the test kit Vitrotest EBV EBNA-1 IgG was 100 %.

11.2. Accuracy

Intra assay repeatability

Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 32-replicate determinations using 1 lot of the test kit.

Serum No.	OD	Ratio	CV, %
34	1.579	5.03	6.2
52	2.512	8.00	5.4

Inter assay reproducibility

Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 4 ELISA performances during 4 days, in 8-replicate determinations.

Serum No.	OD	Ratio	CV, %
34	1.587	5.06	6.4
52	2.542	8.11	4.6

12. LIMITATIONS OF THE PROCEDURE

A positive result in the test kit Vitrotest EBV EBNA-1 IgG indicates the presence of specific antibodies IgG to EBV nuclear antigen produced by infected organism with EBV and remain at the level of detection throughout life. The presence of the antibodies in infants does not indicate the infection with EBV.

The results of Vitrotest EBV EBNA-1 IgG performed on serum from immunosuppressed patients must be interpreted with caution.

The performance characteristics for this assay have not been established for pediatric specimens.

Samples collected very early in the course of an infection may not have detectable levels of IgG.

For correct diagnosis of EBV infection it is recommended to study the presence of anti-VCA-IgG and anti-VCA-IgM antibodies, for example, with test-kits Vitrotest EBV VCA IgG and Vitrotest EBV VCA IgM.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The physician must interpret the results in light of the patient's history, physical findings and other diagnostic procedures.

13. TROUBLESHOOTING

Possible causes	*Solutions*
	High background in all wells
Contaminated washer	Clean the washer head, then rinse it with 30 % ethanol and distilled water
Low quality water or contaminated water	Use distilled/DI with resistivity ≥ 10 MΩ·cm.
Using contaminated glassware	Use clean glassware
Using chlorine based disinfectants	Use disinfectants without chlorine
Using contaminated tips	Use new tips
Increased time of incubation or temperature regimen was changed	Follow incubation regimen according to instruction for use
	High background in a few wells
TMB solution was added more than once	Add TMB solution once
Pipette shaft was contaminated with conjugate solution	Clean the pipette; pipette the liquids carefully
One the channels of the washer was contaminated	Clean the washer channel, clean the washer
	OD of the positive control below normal
Conjugate solution/TMB solution was prepared improperly or not added	Run ELISA repeatedly, prepared conjugate solution / TMB solution properly

Reduced incubation time in one of the stages	Follow incubation regimen according to the instruction for use
Visual colour intensity of the wells does not correspond to optical density	
The optical beam or another component of the reader is misaligned or malfunctioning	Test the absorbance reader's performance

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Catalogue number


Consult instructions for use

In vitro diagnostic medical device


Manufacturer


Caution


Contains sufficient for <*n*> tests


Temperature limit

Batch code


Use-by date


Date of manufacture


Keep away from sunlight


CE mark

Inst_ EBV_EBNA-1_IgG_EL054-96_V03_ENG
Edition 3rd, 20.12.2022

Vitrotest Europe Sp. z o.o.
Krakowska str., 139-155, 50-428, Wroclaw, Poland
tel.: +48 882 950 379,
e-mail: info@vitrotest.pl, www.vitrotest.pl

Vitrotest EBV EBNA-1 IgG

ASSAY PROCEDURE

Keep all reagents and specimens for at least 30 min at 18-25 °C before use

Dispense 90 µl of SAMPLE DILUENT into the wells of ELISA STRIPS
(brown-green colour)

Dispense 10 µl of controls and samples into the wells in the following order:
A1 – CONTROL +,
B1, C1 – CONTROL CUT-OFF,
D1 – CONTROL –,
E1 and other wells – patient samples
(colour changes from brown-green to blue)

Cover wells with an adhesive film, incubate for 30 min at 37 °C

Rinse the wells 5 times with diluted 1:20 (1+19) washing solution Tween-20 (300 µl per well)

Add 100 µl of CONJUGATE SOLUTION into the wells
(green colour)

Cover wells with an adhesive film, incubate for 30 min at 37 °C

Rinse the wells 5 times with diluted 1:20 (1+19) washing solution Tween-20 (300 µl per well)

Add 100 µl of TMB SOLUTION into the wells

Incubate for 15 min in the dark at 18-25 °C

Add 100 µl of STOP SOLUTION
(colour changes from blue to yellow)

Determine the optical density (OD) at 450/620-695 nm

CALCULATION

$CO = (OD_{\text{CONTROL CUT-OFF 1}} + OD_{\text{CONTROL CUT-OFF 2}})/2;$

$Ratio_{\text{sample}} = OD_{\text{sample}}/CO$

INTERPRETATION

$Ratio_{\text{sample}} > 1.0$	POSITIVE
$0.8 \leq Ratio_{\text{sample}} \leq 1.0$	DOUBTFUL
$Ratio_{\text{sample}} < 0.8$	NEGATIVE

Vitrotest EBV VCA IgG

ELISA test kit for the qualitative and semiquantitative determination of IgG class antibodies to capsid antigen of Epstein-Barr Virus

REF EL053-96

IVD Σ 96

1. INTENDED USE

The test kit Vitrotest EBV VCA IgG is an enzyme linked immunosorbent assay (ELISA) for the qualitative and semiquantitative determination of IgG class antibodies to viral capsid antigen (VCA) of Epstein-Barr virus (EBV) in human serum or plasma.

The test kit might be applied for the ELISA using both automatic pipettes and standard equipment as well as open system automated ELISA analyzers.

2. CLINICAL VALUE

Epstein-Barr virus (EBV), also known as human herpesvirus 4, is the causative agent of diseases such as infectious mononucleosis, Burkitt's lymphoma, nasopharyngeal carcinoma.

EBV acquired in early childhood usually does not cause symptoms. Infection in adolescence or early adulthood often results in clinical IM. Typically, the disease presents as pharyngitis, lymphadenopathy, and fever. In most cases, symptoms resolve within 2–4 weeks, but more than 90% of adults develop a latent B-lymphocyte infection. Approximately 1% of immunocompetent individuals may experience severe complications (hepatitis, myocarditis, splenic rupture, neurological complications). In immunosuppressed individuals, primary EBV infection leads to severe disorders (eg, BL).

For laboratory diagnosis of EBV infection, a polymerase chain reaction and serological methods are used that include a test for the detection of heterophilic antibodies and the detection of specific antibodies by ELISA. The latter method allows not only to establish the fact of infection with EBV, but also the stage of the disease.

The optimal combination of serological tests for the diagnosis of EBV infection involves the detection of IgG and IgM antibodies that are specific for the viral capsid antigen (anti-VCA-IgG and anti-VCA-IgM) and nuclear antigen (anti-EBNA-IgG). Anti-VCA-IgM antibodies appear in the body during an early infection and disappear within 4-12 weeks. IgG to VCA appear later (2-4 weeks after infection), their concentration reaches a maximum level at the initial stages of the disease, gradually decreasing. However, these antibodies are determined throughout life. If antibodies to the viral capsid antigen do not appear, a person is susceptible to EBV infection.

Anti-EBNA-IgG are produced by the body 2-4 months after the onset of symptoms. The concentration of the antibodies is maintained at a high level for a long time or throughout life in the majority of infected people. Simultaneous detection of specific IgG to EBNA and VCA indicates the past infection.

3. PRINCIPLE OF THE TEST

Vitrotest EBV VCA IgG ELISA is a solid phase, indirect ELISA method for the determination of IgG antibodies to EBV capsid antigen (VCA) of in a two-step incubation procedure. Microwells are coated with EBV recombinant capsid antigen. During the first incubation step, the specific antibodies to VCA, if present, will be bound to the solid phase precoated antigens. The wells are washed to remove unbound antibodies, leaving only the specific antigen-antibody complexes. A secondary antibody (anti-IgG) which are conjugated to horseradish peroxidase (HRP) added next and bind to the immune complexes on the solid phase. Unbound components are removed by washing. Immune complexes are revealed by addition of chromogen solution containing 3,3',5,5'- tetramethylbenzidine (TMB) and hydrogen peroxide. After 15 minutes the reaction has been stopped, the absorbance values are read using a spectrophotometer at 450/620-695 nm. The colour intensity is proportional to the amount of antibody in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Composition of the test kit

ELISA STRIPS	1x96 wells	Microplate ELISA (12 strips x 8 wells) Each well is coated with EBV recombinant capsid antigen. The wells can be separated.
CONTROL +	1x0.5 ml	Positive control Solution of specific monoclonal immunoglobulins with preservative (pink).
CONTROL –	1x0.5 ml	Negative control Buffer solution with detergent and preservative (yellow).
CONTROL CUT-OFF	1x0.5 ml	Cut-off control Solution of specific monoclonal immunoglobulins with preservative (orange).

SAMPLE DILUENT	1x12 ml	Sample diluent Buffer solution with detergent and preservative (brown-green).
CONJUGATE SOLUTION	1x12 ml	Conjugate solution Buffer solution of monoclonal antibodies to human IgG conjugated to HRP with stabilizers and preservative (green), ready to use.
TMB SOLUTION	1x12 ml	TMB solution TMB, H_2O_2, stabilizers, preservative (colourless), ready to use.
WASH TWEEN 20X	1x50 ml	Washing solution Tw20 (20x concentrate) 20X concentrated of PBS buffer with Tween-20 and NaCl (colourless).
STOP SOLUTION	1x12 ml	Stop solution 0.5 mol/l H_2SO_4 (colourless), ready to use.

Adhesive films (2), sera identification plan (1), instruction for use and certificate of analysis.

4.2. Material required but not provided

- variable volume automatic pipettes (10 µl–1000 µl) and disposable pipette tips;
- plate reader (single wavelength 450 nm or dual wavelength 450/620–695 nm);
- volumetric laboratory glassware (10–1000 ml);
- distilled/DI water;
- incubator thermostatically controlled at 37 °C;
- automatic/semiautomatic plate washer;
- appropriate waste containers for potentially contaminated materials;
- timer;
- absorbent paper;
- disposable gloves;
- disinfectants;
- protective clothes.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

The ELISA assays are time and temperature sensitive. Strictly follow the test procedure and do not modify it.

- do not use expired reagents;
- do not use for analyses and do not mix reagents from different lots or from test kits of different nosology as well as other manufacturer's reagents with Vitrotest kits;

Note: it is possible to use WASH TWEEN 20X, TMB SOLUTION *and* STOP SOLUTION *from other Vitrotest ELISA kits.*

- close reagents after use only with appropriate caps;
- control the filling and full aspiration of the solution in the wells;
- use a new tip for each sample and reagent;
- avoid exposure of kit reagents to direct sunlight;
- TMB SOLUTION must be colourless before use. If TMB SOLUTION is blue or yellow it cannot be used. Avoid any contact of TMB SOLUTION with metals or metal ions. Use glassware thoroughly washed and rinsed with distilled/DI water;
- never use the same glassware for CONJUGATE SOLUTION and TMB SOLUTION.

The manufacturer is not responsible or liable for any incorrect results and/or incidents taking place as a result of any violation of the instruction. The manufacturer is not responsible for visual readings of samples (without using a plate reader).

5.2. Safety

- all components of test kit are intendent for *in vitro* diagnostic use only;
- all materials of human or animal origin should be regarded and handled as potentially infectious;
- the ELISA is only designed for qualified personnel;
- disposable gloves and safety glasses must be worn at all times while performing analysis;
- never eat, drink, smoke or apply cosmetics in the assay laboratory;
- never pipette solutions by mouth;
- controls do not contain of human origin components;
- avoid contact with STOP SOLUTION containing 0.5 mol/l H_2SO_4. It may cause skin irritation and burns;
- some components of the test kit contain low concentrations of harmful compounds and could cause irritation of the skin and the mucosa. In the case of contact of TMB SOLUTION,

STOP SOLUTION or CONJUGATE SOLUTION with skin or mucosa, the place of contact should be immediately rinsed with large amounts of water;
- in case of spilling of solutions that do not contain acid, e.g. sera, rinse the surface with disinfectant, then dry it with absorbent paper. In other case acid first must be neutralized by sodium bicarbonate and then wiped out as described above;
- for information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request.

5.3. Waste treatment

Patient specimens, controls, and incubated microplate strips should be treated as infectious waste, residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

6. STORAGE AND STABILITY

Reagents are stable until stated expiration date on the label when stored refrigerated (2-8 °C). Do not freeze. The kit should be shipped at 2-8 °C. Single transportation at the temperature up to 23 °C for two days is acceptable.

After the first opening of the packaging, the components of the ELISA kits are stable within 3 months, except for those specified in p. 8 of this Instruction.

7. SPECIMEN COLLECTION

The fresh serum or plasma (EDTA, lithium-heparin, sodium citrate, potassium fluoride) samples can be stored for 3 days at 2-8 °C or frozen for longer periods at -20 – -70 °C. Frozen samples must be thawed and kept at room temperature for at least 30 min before use. Do not use preheated samples. Mix thawed samples thoroughly to homogeneity. Avoid repeated freezing/thawing. Samples containing aggregates must be clarified by centrifugation (3000 rpm for 10-15 min). Do not use hyperlipeamic, hyperhaemolysed or contaminated by microorganisms serum specimens. The presence of bilirubin up to concentration of 0.21 mg/ml (361.8 µmol/l), haemoglobin up to concentration of 10 mg/ml and triglycerides up to concentration of 10 mg/ml (11.3 mmol/l) are allowed.

8. REAGENT PREPARATION

It is very important to keep all test components for at least 30 min at room temperature (18-25 ºC) before the assay!

8.1. ELISA STRIPS preparation

Before opening the bag with ELISA STRIPS, keep it at room temperature for 30 min to avoid water condensation inside the wells. Open the vacuum bag and take out the necessary number of the wells. Once opened the bag with the remaining strips and desiccant must be ***resealed with zip-lock*** immediately and kept refrigerated at 2-8 °C for no more than 3 months.

8.2. Washing solution preparation

Check the WASH TWEEN 20X for the presence of salt crystals. If crystals have formed, re-solubilise by warming at 37 °C, until crystals have been fully dissolved (15-20 min). Dilute the WASH TWEEN 20X 1:20 (1+19) with distilled/DI water before use and mix. For example, 4 ml concentrate + 76 ml water is sufficient for 8 wells. Once diluted it is stable at 2-8 °C for 7 days.

9. ASSAY PROCEDURE

9.1. Take out from the protective bag the support frame and the necessary number of the wells ELISA STRIPS (the number of specimens + 4 for controls). Place the wells into the frame. Wells with the controls must be included in every test.
9.2. Complete the sera identification plan.
9.3. Prepare washing solution (see 8.2.).
9.4. Dispense 90 µl of SAMPLE DILUENT into each well.
9.5. Dispense 10 µl of controls and patient samples into the wells in the following order: A1 – CONTROL +, B1, C1 – CONTROL CUT-OFF, and D1 – CONTROL –, other wells – patient samples. Mix gently to avoid foaming. The colour of the sample diluent changes from brown -green to blue.
9.6. Cover strips with an adhesive film and incubate for 30 min at 37 °C.
9.7. At the end of the incubation period, remove and discard the adhesive film and wash the well 5 times with automatic washer or 8-channel pipette as follows:
- aspirate the contents of all wells into a liquid waste container and add immediately a minimum of 300 µl of diluted washing solution to each well;
- soak each well for 30 s between each wash cycle;
- aspirate again. The residual volume must be lower than 5 µl.
- repeat the washing step 4 times;
- after the final washing cycle, turn down the plate onto an absorbent paper and tap it to remove any residual buffer.

9.8. Dispense 100 µl of CONJUGATE SOLUTION per well. Cover strips with a new adhesive film, incubate for 30 min at 37°C.
9.9. At the end of the incubation period, remove and discard the adhesive film and wash the wells five times as described above (see 9.7).
9.10. Dispense 100 µl TMB SOLUTION into all wells. Do not touch the walls and bottoms of the wells to avoid contamination.
9.11. Incubate the strips for 15 min at room temperature (18-25 °C) in the dark. Do not use adhesive film in this step.
9.12. Dispense 100 µl STOP SOLUTION into all wells in the same order and at the same rate as for TMB SOLUTION.
9.13. Read the optical density (OD) of the wells at 450/620-695 nm using a microplate reader within 5 min after adding the STOP SOLUTION. Pay attention to the cleanness of the plate bottom and absence of bubbles in the wells before reading.

Measurement in the single-wave procedure at 450 nm is possible. Reserve blank well to adjust spectrophotometer in such analysis. Only TMB SOLUTION *and* STOP SOLUTION *must be added in blank well.*

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Validation of the test

The test run may be considered valid provided the following criteria are met:

CONTROL +	OD ≥ 1.2
CONTROL CUT-OFF	OD in a range 0.25-0.65
CONTROL –	OD ≤ 0.150

If one of the control cut-off absorbances does not match the above criteria, this value should be discarded and a mean value should be calculated using the remaining cut-off value. If both control cut-off absorbance do not meet the criteria, the test is invalid and must be re-tested.

10.2. Calculation of results

The cut-off (CO) is the mean optical density (OD) of the wells containing CONTROL CUT-OFF:

$$CO = (OD_{CONTROL\ CUT\text{-}OFF\ 1} + OD_{CONTROL\ CUT\text{-}OFF\ 2})/2;$$

The sample result is reported as a Ratio:

$$Ratio_{sample} = OD_{sample}/CO, \quad OD_{sample} – \text{optical density of the well containing sample}$$

10.3. Interpretation of results

$Ratio_{sample} > 1.1$	POSITIVE
$0.9 \leq Ratio_{sample} \leq 1.1$	DOUBTFUL*
$Ratio_{sample} < 0.9$	NEGATIVE

* If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

11. PERFORMANCE CHARACTERISTICS

11.1. Specificity and sensitivity

In the comparative studies of the test kit Vitrotest EBV VCA IgG with other CE marked ELISA kit 162 serum samples that contained IgG antibodies to EBV capsid antigen and 124 serum samples that did not contain serological markers of the EBV infection. Relative sensitivity of the test kit Vitrotest EBV VCA IgG was 99.4 %, relative specificity was 100 %.

11.2. Accuracy

Intra assay repeatability

Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 32-replicate determinations using 1 lot of the test kit.

Serum No.	OD	Ratio	CV, %
9S	2.525	8.02	3.2
22S	1.009	3.20	3.9

Inter assay reproducibility

Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 4 ELISA performances during 4 days, in 8-replicate determinations.

Serum No.	OD	Ratio	CV, %
9S	2.547	8.07	3.8
22S	1.005	3.18	3.7

12. LIMITATIONS OF THE PROCEDURE

A positive result in the test kit Vitrotest EBV VCA IgG indicates the presence of specific antibodies IgG to EBV VCA produced by infected organism with EBV and remain at the level of detection throughout life. The presence of the antibodies in infants does not indicate the infection with EBV.

For correct diagnosis of EBV infection it is recommended to study the presence of anti-VCA-IgM and anti-EBNA-IgG antibodies, for example, with test kits Vitrotest EBV VCA IgM and Vitrotest EBV EBNA-1 IgG.

Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis, in fact, should take into consideration both the results of laboratory tests and the clinical symptoms of the disease.

13. TROUBLESHOOTING

Possible causes	*Solutions*
High background in all wells	
Contaminated washer	Clean the washer head, then rinse it with 30 % ethanol and distilled water
Low quality water or contaminated water	Use distilled/DI with resistivity ≥ 10 MΩ·cm.
Using contaminated glassware	Use clean glassware
Using chlorine based disinfectants	Use disinfectants without chlorine
Using contaminated tips	Use new tips
Increased time of incubation or temperature regimen was changed	Follow incubation regimen according to instruction for use
High background in a few wells	
TMB solution was added more than once	Add TMB solution once
Pipette shaft was contaminated with conjugate solution	Clean the pipette; pipette the liquids carefully
One the channels of the washer was contaminated	Clean the washer channel, clean the washer
OD of the positive control below normal	
Conjugate solution/TMB solution was prepared improperly or not added	Run ELISA repeatedly, prepared conjugate solution / TMB solution properly
Reduced incubation time in one of the stages	Follow incubation regimen according to the instruction for use
Visual colour intensity of the wells does not correspond to optical density	
The optical beam or another component of the reader is misaligned or malfunctioning	Test the absorbance reader's performance

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Catalogue number


Consult instructions for use

In vitro diagnostic medical device


Manufacturer


Caution


Contains sufficient for <*n*> tests


Temperature limit

Batch code


Use-by date


Date of manufacture


Keep away from sunlight


Signifies European conformity (CE) mark

Inst_EBV_VCA_IgG_EL053-96_V01_ENG
Edition 1st, 05.01.2022

Vitrotest Europe Sp. z o.o.
Krakowska str., 139-155, 50-428, Wroclaw, Poland
tel.: +48 882 950 379,
e-mail: info@vitrotest.pl, www.vitrotest.pl

Vitrotest EBV VCA IgG

ASSAY PROCEDURE

Keep all reagents and specimens for at least 30 min at 18-25 °C before use

Dispense 90 µl of SAMPLE DILUENT into the wells of ELISA STRIPS
(brown-green colour)

Dispense 10 µl of controls and samples into the wells in the following order:
A1 – CONTROL +,
B1, C1 – CONTROL CUT-OFF,
D1 – CONTROL –,
E1 and other wells – patient samples
(colour changes from brown-green to blue)

Cover wells with an adhesive film, incubate for 30 min at 37 °C

Rinse the wells 5 times with diluted 1:20 (1+19) washing solution Tween-20 (300 µl per well)

Add 100 µl of CONJUGATE SOLUTION into the wells
(green colour)

Cover wells with an adhesive film, incubate for 30 min at 37 °C

Rinse the wells 5 times with diluted 1:20 (1+19) washing solution Tween-20 (300 µl per well)

Add 100 µl of TMB SOLUTION into the wells

Incubate for 15 min in the dark at 18-25 °C

Add 100 µl of STOP SOLUTION
(colour changes from blue to yellow)

Determine the optical density (OD) at 450/620-695 nm

CALCULATION

$CO = (OD_{CONTROL\ CUT\text{-}OFF\ 1} + OD_{CONTROL\ CUT\text{-}OFF\ 2})/2;$

$Ratio_{sample} = OD_{sample}/CO$

INTERPRETATION

$Ratio_{sample} > 1.1$	POSITIVE
$0.9 \leq Ratio_{sample} \leq 1.1$	DOUBTFUL
$Ratio_{sample} < 0.9$	NEGATIVE

Vitrotest EBV VCA IgM

ELISA test kit for the qualitative and semiquantitative determination of IgM class antibodies to capsid antigen of Epstein-Barr Virus

REF EL052-96

IVD Σ 96

1. INTENDED USE

The test kit Vitrotest EBV VCA IgM is an enzyme linked immunosorbent assay (ELISA) for the qualitative and semiquantitative determination of IgM class antibodies to viral capsid antigen (VCA) of Epstein-Barr virus (EBV) in human serum or plasma. The test kit might be applied for the ELISA using both automatic pipettes and standard equipment as well as open system automated ELISA analyzers.

2. CLINICAL VALUE

Epstein-Barr virus (EBV), also known as human herpesvirus 4, is the causative agent of diseases such as infectious mononucleosis, Burkitt's lymphoma, nasopharyngeal carcinoma.

EBV acquired in early childhood usually does not cause symptoms. Infection in adolescence or early adulthood often results in clinical IM. Typically, the disease presents as pharyngitis, lymphadenopathy, and fever. In most cases, symptoms resolve within 2–4 weeks, but more than 90% of adults develop a latent B-lymphocyte infection. Approximately 1% of immunocompetent individuals may experience severe complications (hepatitis, myocarditis, splenic rupture, neurological complications). In immunosuppressed individuals, primary EBV infection leads to severe disorders (eg, BL).

For laboratory diagnosis of EBV infection, a polymerase chain reaction and serological methods are used that include a test for the detection of heterophilic antibodies and the detection of specific antibodies by ELISA. The latter method allows not only to establish the fact of infection with EBV, but also the stage of the disease.

The optimal combination of serological tests for the diagnosis of EBV infection involves the detection of IgG and IgM antibodies that are specific for the viral capsid antigen (anti-VCA-IgG and anti-VCA-IgM) and nuclear antigen (anti-EBNA-IgG). Anti-VCA-IgM antibodies appear in the body during an early infection and disappear within 4-12 weeks. IgG to VCA appear later (2-4 weeks after infection), their concentration reaches a maximum level at the initial stages of the disease, gradually decreasing. However, these antibodies are determined throughout life. If antibodies to the viral capsid antigen do not appear, a person is susceptible to EBV infection.

Anti-EBNA-IgG are produced by the body 2-4 months after the onset of symptoms. The concentration of the antibodies is maintained at a high level for a long time or throughout life in the majority of infected people. Simultaneous detection of specific IgG to EBNA and VCA indicates the past infection.

3. PRINCIPLE OF THE TEST

Determination of IgM antibodies to EBV VCA in the test kit Vitrotest EBV VCA IgM is based on a solid phase, indirect «IgM-capture» ELISA in a two-step incubation procedure. Microwells are coated with anti-IgM monoclonal antibodies. During the first incubation step, IgM antibodies, if present, will be captured by the solid phase precoated monoclonal antibodies. The wells are washed to remove unbound antibodies. A recombinant capsid antigen of EBV, which is conjugated to horseradish peroxidase (HRP), is added next and binds to the anti-VCA IgM on the solid phase. Unbound components are removed by washing. Immune complexes are revealed by addition of chromogen solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide. After 15 minutes the reaction has been stopped, the absorbance values are read using a spectrophotometer at 450/620-695 nm. The colour intensity is proportional to the amount of antibody in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Composition of the test kit

ELISA STRIPS	1x96 wells	Microplate ELISA (12 strips x 8 wells) Each well is coated with monoclonal anti-IgM antibodies. The wells can be separated.
CONTROL +	1x0.5 ml	Positive control Solution of specific monoclonal immunoglobulins with preservative (pink).
CONTROL –	1x0.5 ml	Negative control Buffer solution with detergent and preservative (yellow).
CONTROL CUT-OFF	1x0.5 ml	Cut-off control Solution of specific monoclonal immunoglobulins with preservative (orange).

CONJUGATE 11X	1x1.3 ml	**Conjugate (11X)** 11-fold concentrate of recombinant Epstein-Barr virus capsid antigen (VCA) conjugated to HRP in a buffer solution with stabilizers (blue).
SAMPLE DILUENT	1x12 ml	**Sample diluent** Buffer solution with detergent and preservative (violet).
CONJUGATE DILUENT	1x13 ml	**Conjugate diluent** Buffer solution with detergent and preservative (yellow).
TMB SOLUTION	1x12 ml	**TMB solution** TMB, H_2O_2, stabilizers, preservative (colourless), ready to use.
WASH TWEEN 20X	1x50 ml	**Washing solution Tw20 (20x concentrate)** 20X concentrated of PBS buffer with Tween-20 and NaCl (colourless).
STOP SOLUTION	1x12 ml	**Stop solution** 0.5 mol/l H_2SO_4 (colourless), ready to use.

Adhesive films (2), sera identification plan (1), instruction for use and certificate of analysis.

4.2. Material required but not provided

- variable volume automatic pipettes (10 µl–1000 µl) and disposable pipette tips;
- plate reader (single wavelength 450 nm or dual wavelength 450/620–695 nm);
- volumetric laboratory glassware (10–1000 ml);
- distilled/DI water;
- incubator thermostatically controlled at 37 °C;
- automatic/semiautomatic plate washer;
- appropriate waste containers for potentially contaminated materials;
- timer;
- absorbent paper;
- disposable gloves;
- disinfectants;
- protective clothes.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

The ELISA assays are time and temperature sensitive. Strictly follow the test procedure and do not modify it.

- do not use expired reagents;
- do not use for analyses and do not mix reagents from different lots or from test kits of different nosology as well as other manufacturer's reagents with Vitrotest kits;

Note: it is possible to use WASH TWEEN 20X, TMB SOLUTION *and* STOP SOLUTION *from other Vitrotest ELISA kits.*

- close reagents after use only with appropriate caps;
- control the filling and full aspiration of the solution in the wells;
- use a new tip for each sample and reagent;
- avoid exposure of kit reagents to direct sunlight;
- TMB SOLUTION must be colourless before use. If TMB SOLUTION is blue or yellow it cannot be used. Avoid any contact of TMB SOLUTION with metals or metal ions. Use glassware thoroughly washed and rinsed with distilled/DI water;
- never use the same glassware for conjugate solution and TMB SOLUTION.

The manufacturer is not responsible or liable for any incorrect results and/or incidents taking place as a result of any violation of the instruction. The manufacturer is not responsible for visual readings of samples (without using a plate reader).

5.2. Safety

- all components of test kit are intendent for *in vitro* diagnostic use only;
- all materials of human or animal origin should be regarded and handled as potentially infectious;
- the ELISA is only designed for qualified personnel;
- disposable gloves and safety glasses must be worn at all times while performing analysis;
- never eat, drink, smoke or apply cosmetics in the assay laboratory;
- never pipette solutions by mouth;
- controls do not contain of human origin components;

– avoid contact with STOP SOLUTION containing 0.5 mol/l H_2SO_4. It may cause skin irritation and burns;
– some components of the test kit contain low concentrations of harmful compounds and could cause irritation of the skin and the mucosa. In the case of contact of TMB SOLUTION, STOP SOLUTION or conjugate solution with skin or mucosa, the place of contact should be immediately rinsed with large amounts of water;
– in case of spilling of solutions that do not contain acid, e.g. sera, rinse the surface with disinfectant, then dry it with absorbent paper. In other case acid first must be neutralized by sodium bicarbonate and then wiped out as described above;
– for information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request.

5.3. Waste treatment

Patient specimens, controls, and incubated microplate strips should be treated as infectious waste, residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

6. STORAGE AND STABILITY

Reagents are stable until stated expiration date on the label when stored refrigerated (2-8 °C). Do not freeze. The kit should be shipped at 2-8 °C. Single transportation at the temperature up to 23 °C for two days is acceptable.

After the first opening of the packaging, the components of the ELISA kits are stable within 3 months, except for those specified in p. 8 of this Instruction.

7. SPECIMEN COLLECTION

The fresh serum or plasma (EDTA, lithium-heparin, sodium citrate, potassium fluoride) samples can be stored for 3 days at 2-8 °C or frozen for longer periods at -20 – -70 °C. Frozen samples must be thawed and kept at room temperature for at least 30 min before use. Do not use preheated samples. Mix thawed samples thoroughly to homogeneity. Avoid repeated freezing/thawing. Samples containing aggregates must be clarified by centrifugation (3000 rpm for 10-15 min). Do not use hyperlipeamic, hyperhaemolysed or contaminated by microorganisms serum specimens. The presence of bilirubin up to concentration of 0.21 mg/ml (361.8 µmol/l), haemoglobin up to concentration of 10 mg/ml and triglycerides up to concentration of 10 mg/ml (11.3 mmol/l) are allowed.

8. REAGENT PREPARATION

It is very important to keep all test components for at least 30 min at room temperature (18-25 ºC) before the assay!

8.1. ELISA STRIPS preparation

Before opening the bag with ELISA STRIPS, keep it at room temperature for 30 min to avoid water condensation inside the wells. Open the vacuum bag and take out the necessary number of the wells. Once opened the bag with the remaining strips and desiccant must be ***resealed with zip-lock*** immediately and kept refrigerated at 2-8 °C for no more than 3 months.

8.2. Washing solution preparation

Check the WASH TWEEN 20X for the presence of salt crystals. If crystals have formed, re-solubilise by warming at 37 °C, until crystals have been fully dissolved (15-20 min). Dilute the WASH TWEEN 20X 1:20 (1+19) with distilled/DI water before use and mix. For example, 4 ml concentrate + 76 ml water is sufficient for 8 wells. Once diluted it is stable at 2-8 °C for 7 days.

8.3. Conjugate solution preparation

Conjugate working solution is prepared as follows:

Dilute the CONJUGATE 11X (blue) in clean vial with the CONJUGATE DILUENT 1:11 (1 + 10). The colour of the solution changes to green. For example, for 8 wells of the analysis 100 µl of the CONJUGATE 11X add to 1 ml of the CONJUGATE DILUENT. Once diluted it is stable at 2-8°C for 1 day.

9. ASSAY PROCEDURE

9.1. Take out from the protective bag the support frame and the necessary number of the wells ELISA STRIPS (the number of specimens + 4 for controls). Place the wells into the frame. Wells with the controls must be included in every test.
9.2. Complete the sera identification plan.
9.3. Prepare washing solution (see 8.2.).
9.4. Dispense 90 µl of SAMPLE DILUENT into each well.
9.5. Dispense 10 µl of controls and patient samples into the wells in the following order: A1 – CONTROL +, B1, C1 – CONTROL CUT-OFF, and D1 – CONTROL –, other wells – patient samples. Mix gently to avoid foaming. The colour of the sample diluent changes from violet to blue.
9.6. Cover strips with an adhesive film and incubate for 30 min at 37 °C.

9.7. At the end of the incubation period, remove and discard the adhesive film and wash the well 5 times with automatic washer or 8-channel pipette as follows:
- aspirate the contents of all wells into a liquid waste container and add immediately a minimum of 300 µl of diluted washing solution to each well;
- soak each well for 30 s between each wash cycle;
- aspirate again. The residual volume must be lower than 5 µl.
- repeat the washing step 4 times;
- after the final washing cycle, turn down the plate onto an absorbent paper and tap it to remove any residual buffer.

9.8. Prepare conjugate solution according to 8.3.
9.9. Dispense 100 µl of the diluted conjugate solution (1:11) per well. Cover strips with a new adhesive film, incubate for 30 min at 37°C.
9.10. At the end of the incubation period, remove and discard the adhesive film and wash the wells five times as described above (see 9.7).
9.11. Dispense 100 µl TMB SOLUTION into all wells. Do not touch the walls and bottoms of the wells to avoid contamination.
9.12. Incubate the strips for 15 min at room temperature (18-25 °C) in the dark. Do not use adhesive film in this step.
9.13. Dispense 100 µl STOP SOLUTION into all wells in the same order and at the same rate as for TMB SOLUTION.
9.14. Read the optical density (OD) of the wells at 450/620-695 nm using a microplate reader within 5 min after adding the STOP SOLUTION. Pay attention to the cleanness of the plate bottom and absence of bubbles in the wells before reading.

Measurement in the single-wave procedure at 450 nm is possible. Reserve blank well to adjust spectrophotometer in such analysis. Only TMB SOLUTION *and* STOP SOLUTION *must be added in blank well.*

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Validation of the test

The test run may be considered valid provided the following criteria are met:

CONTROL +	OD ≥ 1.2
CONTROL CUT-OFF	OD in a range 0.25-0.65
CONTROL –	OD ≤ 0.150

If one of the control cut-off absorbances does not match the above criteria, this value should be discarded and a mean value should be calculated using the remaining cut-off value. If both control cut-off absorbance do not meet the criteria, the test is invalid and must be re-tested.

10.2. Calculation of results

The cut-off (CO) is the mean optical density (OD) of the wells containing CONTROL CUT-OFF:

$$CO = (OD_{\text{CONTROL CUT-OFF 1}} + OD_{\text{CONTROL CUT-OFF 2}})/2;$$

The sample result is reported as a Ratio:

$$Ratio_{sample} = OD_{sample}/CO, \quad OD_{sample} - \text{optical density of the well containing sample}$$

10.3. Interpretation of results

$Ratio_{sample} > 1.1$	POSITIVE
$0.9 \leq Ratio_{sample} \leq 1.1$	DOUBTFUL*
$Ratio_{sample} < 0.9$	NEGATIVE

* If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

11. PERFORMANCE CHARACTERISTICS

11.1. Specificity and sensitivity

In the comparative studies of the test kit Vitrotest EBV VCA IgM with other CE marked ELISA kit 102 serum samples that contained IgM antibodies to EBV capsid antigen and 124 serum samples that did not contain serological markers of the EBV infection. Relative sensitivity of the test kit Vitrotest EBV VCA IgM was 99.0 %, relative specificity was 99.2 %.

11.2. Accuracy

Intra assay repeatability

Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 32-replicate determinations using 1 lot of the test kit.

Serum No.	OD	Ratio	CV, %
1100	1.468	4.42	6.9
1843	0.753	2.27	6.0

Inter assay reproducibility

Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 4 ELISA performances during 4 days, in 8-replicate determinations.

Serum No.	OD	Ratio	CV, %
1100	1.533	4.65	6.4
1843	0.806	2.44	6.1

12. LIMITATIONS OF THE PROCEDURE

A positive result in the test kit Vitrotest EBV VCA IgM indicates the presence of specific antibodies IgM to EBV VCA. IgM antibodies specific to VCA present at the acute EBV infection. IgM antibodies do not always indicate the primary infection but recurrent infection as well.

It is impossible to completely eliminate the cross-reactivity of IgM antibodies to cytomegalovirus because both the herpesviruses have similar antigenic determinants. To exclude false positive results it is recommended to investigate the serological profiles in the dynamics (CMV IgG, CMV IgM, VCA IgG, VCA IgM and EBNA IgG), detect viral DNA in the samples and take into account clinical symptoms of the disease.

In case of obtaining sample after a little time after the infection IgM antibodies may not be detected. Depending on the clinical symptoms it is necessary to retest the samples obtained in 2-4 weeks.

The results obtained for immunosuppressed individuals should be interpreted with the precautions.

For correct diagnosis of EBV infection it is recommended to study the presence of anti-VCA-IgG and anti-EBNA-IgG antibodies, for example, with test kits Vitrotest EBV VCA IgG and Vitrotest EBV EBNA-1 IgG.

Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis, in fact, should take into consideration both the results of laboratory tests and the clinical symptoms of the disease.

13. TROUBLESHOOTING

Possible causes	*Solutions*
High background in all wells	
Contaminated washer	Clean the washer head, then rinse it with 30 % ethanol and distilled water
Low quality water or contaminated water	Use distilled/DI with resistivity ≥ 10 MΩ·cm.
Using contaminated glassware	Use clean glassware
Using chlorine based disinfectants	Use disinfectants without chlorine
Using contaminated tips	Use new tips
Increased time of incubation or temperature regimen was changed	Follow incubation regimen according to instruction for use
High background in a few wells	
TMB solution was added more than once	Add TMB solution once
Pipette shaft was contaminated with conjugate solution	Clean the pipette; pipette the liquids carefully
One the channels of the washer was contaminated	Clean the washer channel, clean the washer

OD of the positive control below normal	
Conjugate solution/TMB solution was prepared improperly or not added	Run ELISA repeatedly, prepared conjugate solution / TMB solution properly
Reduced incubation time in one of the stages	Follow incubation regimen according to the instruction for use
Visual colour intensity of the wells does not correspond to optical density	
The optical beam or another component of the reader is misaligned or malfunctioning	Test the absorbance reader's performance

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Catalogue number


Consult instructions for use

In vitro diagnostic medical device


Manufacturer


Caution


Contains sufficient for <*n*> tests


Temperature limit

Batch code


Use-by date


Date of manufacture


Keep away from sunlight


Signifies European conformity (CE) mark

Inst_EBV_VCA_IgM_EL052-96_V01_ENG
Edition 1st, 05.01.2022

Vitrotest Europe Sp. z o.o.
Krakowska str., 139-155, 50-428, Wroclaw, Poland
tel.: +48 882 950 379,
e-mail: info@vitrotest.pl, www.vitrotest.pl

Vitrotest EBV VCA IgM

ASSAY PROCEDURE

Keep all reagents and specimens for at least 30 min at 18-25 °C before use

Dispense 90 µl of SAMPLE DILUENT into the wells of ELISA STRIPS
(violet colour)

Dispense 10 µl of controls and samples into the wells in the following order:
A1 – CONTROL +,
B1, C1 – CONTROL CUT-OFF,
D1 – CONTROL –,
E1 and other wells – patient samples
(colour changes from violet to blue)

Cover wells with an adhesive film, incubate for 30 min at 37 °C

Rinse the wells 5 times with diluted 1:20 (1+19) washing solution Tween-20 (300 µl per well)

Add 100 µl of diluted (1:11) conjugate solution to each well
(green colour)

Cover wells with an adhesive film, incubate for 30 min at 37 °C

Rinse the wells 5 times with diluted 1:20 (1+19) washing solution Tween-20 (300 µl per well)

Add 100 µl of TMB SOLUTION into the wells

Incubate for 15 min in the dark at 18-25 °C

Add 100 µl of STOP SOLUTION
(colour changes from blue to yellow)

Determine the optical density (OD) at 450/620-695 nm

CALCULATION

$CO = (OD_{CONTROL\ CUT\text{-}OFF\ 1} + OD_{CONTROL\ CUT\text{-}OFF\ 2})/2;$

$Ratio_{sample} = OD_{sample}/CO$

INTERPRETATION

$Ratio_{sample} > 1.1$	POSITIVE
$0.9 \leq Ratio_{sample} \leq 1.1$	DOUBTFUL
$Ratio_{sample} < 0.9$	NEGATIVE