Version 27-05/24

User's Manual

VZV IgG ELISA Kit

REF **DEIA472**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The kit is intended for professional use for the qualitative and semiquantitative detection of IgG antibodies to varicella zoster virus (VZV) in human serum and plasma and for the determination of intrathecal synthesis in cerebrospinal fluid. The test is also useful in the diagnosis of VZV-associated diseases, namely varicella and herpes zoster. The test can also be utilized for the differential detection of neuroinfections (encephalitis, meningitis, cerebellitis, vasculitis, myelitis and inflammatory neuropathies), infections of the eye and of exanthematous diseases of the skin.

Principles of Testing

Anti-VZV IgG (CSF) ELISA is a solid-phase immunoanalytical test. A native antigen VZV is bound to the surface of the wells. If antibodies are present in the test samples, they will bind to the immobilized proteins. The bound antibodies then react in the next step with horseradish peroxidase-labeled anti-human IgG antibodies. The amount of bound labeled antibodies is determined by a color enzymatic reaction. Negative samples do not react, a slight change in the color of the wells is the background of the reaction. Intrathecal antibody testing provides information on the anti-VZV antibody response in the central nervous system. For this assay, it is necessary to quantify the concentration of VZV-specific IgG antibodies in paired serum / plasma and cerebrospinal fluid samples taken from the patient at the same time. Accurate quantification of antibodies can only be performed according to the linear part of the calibration curve. Therefore, it is recommended to test the serum in two dilutions (Reagent preparation). The total IgG and albumin concentrations in both samples must be known for the determination. The calculation of intrathecal synthesis of specific antibodies is performed using the Reiber equation (see Intrathecal synthesis of specific IgG antibodies).

Reagents And Materials Provided

1. ELISA break-away strips in the handling frame coated with the specific antigen STRIPS Ag, 1 × 12 pcs

2. 1.3 mL Standard A = Negative control human serum, r.t.u. [1)] ST A/NC, 1 vial

3. 1.3 mL Standard B (human serum), r.t.u. ST B, 1 vial

4. 1.3 mL Standard C (human serum), r.t.u. ST C, 1 vial

5. 2.0 mL Standard D = Calibrator (human serum), r.t.u. ST D/CAL, 1 vial

6. 1.3 mL Standard E = Positive control human serum, r.t.u. ST E/PC, 1 vial

7. 13 mL Anti-human IgG animal antibodies labelled with horseradish peroxidase (anti-IgG Px conjugate) r.t.u. CONJ, 1 vial

8. 55 mL Wash buffer, 10× concentrated WASH 10×, 1 vial

9. 60 mL Dilution buffer, r.t.u. DIL, 1 vial

10. 13 mL Chromogenic substrate TMB, r.t.u. (TMB/H_2O_2) TMB, 1 vial

11. 13 mL Stop solution, r.t.u. (0.4 M sulfuric acid) STOP, 1 vial

12. Instruction manual

13. Quality Control Certificate

[1)] r.t.u., ready to use

Notice: Control sera may be colorless to yellowish or blue due to the use of different diluents.

Materials Required But Not Supplied

Distilled/deionised water for dilution of the Wash buffer WASH 10×, pipetting equipment, equipment for liquid dispensing and strip washing, spectrophotometer/colorimeter, thermostat for incubation of the microtiter plate at 37 °C.

All instruments and devices used must have a valid function validation.

Storage

2°C - 10°C

Reagent Preparation

1. Allow all kit components to reach room temperature. Turn on the thermostat to 37 °C.
2. Thoroughly mix Dilution buffer DIL, Conjugate anti-IgG Px CONJ and Chromogenic substrate TMB.
3. Thoroughly mix tested samples and control sera just prior to testing. Dilute the tested serum/plasma samples 101× with Dilution buffer DIL (e.g. 5 µL sample + 500 µL Dilution buffer DIL). For evaluation of the intrathecal production test two dilutions of serum samples are recommended: 101× and 404×. Dilution 404× prepare by 4× diluting of the 101× diluted serum sample (e.g. 150 µL of Dilution buffer + 50 µL of serum sample diluted 101×). Dilute cerebrospinal fluid samples 1:1 in Dilution buffer (e.g. 75 µL of cerebrospinal fluid sample + 75 µL of Dilution buffer). Do not dilute control sera and calibrator, they are in working concentration (r.t.u., ready to use).
4. Prepare a working concentration of Wash buffer WASH 10x by diluting it 10x in a suitable volume of distilled/deionized water (eg. 50 mL of WASH 10x + 450 mL H_2O). If there are salt crystals in the concentrated solution, warm it in a water bath of + 32 °C to + 37 °C and mix well before diluting. Unused wash solution in working concentration can be stored for 1 month at room temperature.
5. Do not dilute Conjugate anti-IgG Px CONJ, Chromogenic substrate TMB and Stop solution STOP, they are ready to use.

Assay Procedure

The manufacturer is not responsible for the correct function of the kit if the assay procedure is not followed.

1. Allow strips STRIPS Ag, vacuum sealed with desiccant, to reach room temperature before opening the bag, to avoid dew condensation of the plate. Prepare the required number of strips for the reaction. Seal unused strips together with the desiccant in a zipper bag or seal under vacuum.
2. Choose the proper method for data interpretation (see paragraph 8) and apply the samples to the plate

accordingly. Fill the wells with 100 µL of Standards and diluted samples according to the pipetting scheme: Start with filling the first well with Dilution buffer DIL to estimate the reaction background (BLANK). In case of choosing the qualitative or semiquantitative evaluation, fill two wells with Standard D ST D/CAL, serves as a calibrator. Next well with Positive control serum ST E/PC and next one well with Negative control serum ST A/NC (see Figure 1). In case of quantitative evaluation, fill one of each well wilt Standards A-E (ST A/NC, ST B, ST C, ST D/CAL, ST E/PC). Fill the remaining wells with diluted samples (S1, S2, S3, ...), (see Figure 2). It is sufficient to apply samples as singlets, however, if you wish to minimize the laboratory error apply control sera and tested samples as doublets (Figure 3), in the case of qualitative and semiquantitative evaluation apply Standard D ST D/CAL as triplet. We recommend to include positive reference serum sample (your in-house internal control) into each run to follow the sequence, variability and accuracy of calibration. Incubate 30 minutes (+/- 2 min) at 37 °C.

3. Aspirate the contents of the wells into a safety collection bottle containing a suitable disinfectant (see WARNINGS). Then wash the wells 4 times with 250 µL of wash solution. Avoid overflowing the solution out of the wells. Aspirate the contents of the wells and tap the plate on an adsorbent paper.
4. Mix thoroughly the vial of anti-IgG Px conjugate CONJ and pipette 100 µL of anti-IgG Px conjugate CONJ into the wells. Incubate 30 minutes (+/- 2 min) at 37 °C.
5. Aspirate the fluid from the wells and wash them with 4 x 250 µL of wash solution. Aspirate and tap.
6. Pipette 100 µL of Chromogenic substrate TMB solution into the wells. Incubate for 15 minutes (+/- 30 sec) in the dark at room temperature. Start measuring the incubation time after pipetting the first strip of the plate. Follow this rule to avoid breaking the time interval. Pipette quickly at regular rhythm, or use a suitable dispenser. Cover the strips with foil, an opaque lid, or keep them in a dark place for the duration of the reaction.
7. Stop the reaction by adding 100 µL of Stop solution STOP. Pipette at the same rate as the Chromogenic substrate TMB so that the enzymatic reaction proceeds in all wells at the same time. Check that there are no bubbles in the wells, if so, gently tap the plate frame to remove them.
8. Measure the intensity of the colour reaction on a spectrophotometer/colorimeter at 450 nm within 10 minutes after stopping the reaction. We recommend using a 620-690 nm reference filter.

Figure 1: Scheme of sample application for qualitative and semiquantitative evaluation

	1	2	3	4	5	6	7	8	9	10	11	12
a	DIL											
b	ST D/CAL											
c	ST D/CAL											
d	ST E/PC											
e	ST A/NC											
f	S1											
g	S2											
h	S...											

Figure 2: Scheme of sample application for quantitative evaluation (singlets)

	1	2	3	4	5	6	7	8	9	10	11	12
a	DIL	S3										
b	STA/NC	S...										
c	ST B											
d	ST C											
e	STD/CAL											
f	STE/PC											
g	S1											
h	S2											

Figure 3: Scheme of sample application for quantitative evaluation (doublets)

	1	2	3	4	5	6	7	8	9	10	11	12
a	DIL	S2										
b	STA/NC	S2										
c	ST B	S...										
d	ST C	S...										
e	STD/CAL											
f	STE/PC											
g	S1											
h	S1											

9. TEST EVALUATION

   First, subtract the absorbance of the well with Dilution buffer DIL (BLANK = reaction background) from the calibrator, control sera, and test samples. If the values of Control sera or tested samples are negative after background subtraction, consider them as zero value.

Calculation

Qualitative orientation evaluation for sera/plasma samples

1. Calculate the mean OD value of the Standard D ST D/CAL from the two wells. If you are applying three Standard D ST D/CAL wells and some of these values differ by more than 20% from the mean, do not use it for calculation and calculate the mean of the remaining two values.
2. Determine the cut-off value by multiplying the mean OD value of the Standard ST D/CAL by the correction factor. The value of the correction factor is stated in the Quality Control Certificate for the given kit lot.
3. Samples with an OD value < 90 % cut-off are negative and samples with an OD value > 110 % cut-off are considered positive.

Semiquantitative evaluation for sera/plasma samples

Determine Positivity Index for each sample:

1. First determine the cut-off value as in the previous evaluation method (See **Qualitative orientation evaluation for sera/plasma samples** , point 2).
2. Determine the index value for each sample by dividing the OD of the test sample by the cut-off value. Read the appropriate degree of reactivity of the sample (See RESULTS EVALUATION).

RESULTS EVALUATION

Positivity index	Evaluation
< 0.90	Negative
0.90 – 1.10	+/-
> 1.10	Positive*

* on the basis of the Positivity Index value it is possible to estimate semiquantitatively the amount of antibodies in the sample

Example:

Obtained OD Standard D ST D/CAL = 1.807; 1.704; 1.750

Mean OD Standard D ST D/CAL = 1.754

OD sample = 0.800

Correction factor Standard D ST D/CAL = 0.15

Cut-off value = 1.754 x 0.15 = 0.263

Positivity index value = 0.800 / 0.263 = 3.04

Note: A rating of +/- means that the sample is in the gray zone. Repeat the test for this result. If the sample is again in the gray zone after retesting, repeat the test with an alternative method or use a sample from a new sample from the same individual 1-2 weeks later.

Processing of results for Quantitative interpretation

Compute the sample antibody titers in artificial units (mIU/mL) as follows:

1. Construct the calibration curve by plotting the units of Standards (x-axis) (the concentration of each Standards is mentioned in enclosed Quality control certificate) to absorbance (OD) of Standards (y-axis).
2. Find the place where the absorbance of tested samples intersects calibration curve and find the corresponding values (mIU/mL) on the axis x. It is possible to use various softwares for the standard curve fitting and for the calculation of the unknowns, e.g. Winliana, KimQ. For better fitting, the polynomic (fourparameter) function is the most convenient.
3. Calibration curve and units of standard are related to serum diluted 101x. By other dilution of serum or the cerebrospinal fluid you obtained, using the calibration curve, the number of units in the sample (mIU/sample). These units must be converted to the mIU/mL according to this formula:

   (mIU/ sample * dilution of sample) / (101) = mIU/ml

The evaluation in arbitrary units for samples is stated in the Quality Control Certificate.

Note 1: A rating of +/- means that the sample is in the gray zone. Repeat the test for this result. If the sample is again in the gray zone after retesting, repeat the test with an alternative method or use a sample from a new sample from the same individual 1-2 weeks later.

Note 2: Quantification is accurate only in the linear part of the calibration curve. If the measured OD of the sample exceeds the linearity interval (OD 0.100 – 2.400), it is necessary to repeat the testing of the sample at

a higher dilution for accurate quantification.

Intrathecal synthesis of specific IgG antibodies

The determination of IgG concentration against VZV in cerebrospinal fluid (MM) can only be interpreted on the basis of the calculation of intrathecal antibody synthesis.

1. Calculation of antibody index (AI) (according to Reiber)

a. Calculation of the ratio between the concentrations of total IgG ($Q_{total\ IgG}$) and total albumin ($Q_{total\ alb}$) in cerebrospinal fluid and serum. Calculate $Q_{total\ IgG}$ and $Q_{total\ albums}$ according to the formula:

$$Q_{total\ IgG} = \frac{\text{total IgG v MM}}{\text{total IgG v serum}} \qquad Q_{total\ alb} = \frac{\text{total alb v MM}}{\text{total alb v serum}}$$

b. Calculation of the Q_{limIgG} cut-off coefficient, ie the amount of IgG in the cerebrospinal fluid that can come from the systemic circulation under a given barrier state (Reiber's hyperbolic function). Calculate Q_{limIgG} according to the formula:

$$Q_{limIgG} = 0.93 \times \sqrt{(Q_{total\ alb})^2 + 6 \times 10^{-6}} - 1.7 \times 10^{-3}$$

c. Calculation of the coefficient of pathogen-specific IgG antibodies $Q_{path.\text{-}spec.IgG}$, which indicates the ratio between the concentration of specific IgG in cerebrospinal fluid and serum. Calculate $Q_{path.\text{-}spec.IgG}$ according to the formula:

$$Q_{path.\text{-}spec.IgG} = \frac{\text{c spec.IgG MM (mIU/ml)}}{\text{c spec.IgG sérum (mIU/ml)}}$$

where c spec.IgG MM / serum is the determined concentration of specific antibodies in mIU/mL in cerebrospinal fluid / serum multiplied by sample dilution (see Reagent Preparation)

Example:

c spec.IgG MM = 4.512 mIU / mL

c spec.IgG serum = 60 mIU / mL

$Q_{path.\text{-}spec.IgG}$ = 4.512/60

$Q_{path.\text{-}spec.IgG} = 75.2 \times 10^{-3}$

d. Calculation of antibody index AI

a) If: $Q_{total\ IgG} < Q_{limIgG}$, calculate AI according to the formula:

$$AI = \frac{Q_{path.\text{-}spec.IgG}}{Q_{total\ IgG}}$$

b) If: $Q_{total\ IgG} > Q_{limIgG}$, calculate AI according to the formula:

$$AI = \frac{Q_{path.\text{-}spec.IgG}}{Q_{limIgG}}$$

Interpretation Of Results

The test is also useful in the diagnosis of VZV-associated diseases, namely varicella and herpes zoster. The test can also be utilized for the differential diagnosis of neuroinfections (encephalitis, meningitis, cerebellitis, vasculitis, myelitis and inflammatory neuropathies), infections of the eye and of exanthematous diseases of the skin.

Evaluation

Serum antibody	CSF antibody	AI (Antibody Index)	Evaluation
Negative	Regardless of the result	Cannot be calculated	Absent
Grey zone / Positive		< 0.5	Cannot be evaluated
		0.5 – 1.5	Absent
		1.5 – 2.0	Suspect intrathecal synthesis
		> 2.0	Present intrathecal synthesis

Note 1: The calculation of intrathecal synthesis from serum samples with antibodies at cut-off point can be affected by experimental error. In this case, it is recommended to monitor the dynamics of intrathecal synthesis

Note 2: Antibody index cannot be assessed in patients with damaged Blood-Brain Barrier.

Performance Characteristics

The kit is intended for the qualitative, semiquantitative and quantitative detection of anti-VZV IgG antibodies in human serum, plasma and cerebrospinal fluid. Suitable specimens are serum, plasma (heparinised) and cerebrospinal fluid samples obtained by standard laboratory techniques.

Validity of the test

The absorbance value of the Dilution buffer DIL (BLANK = reaction background) is stated in the Quality Control Certificate of the lot. The OD values of the standards / control sera and the ratio of the OD values of the standards ST E/PC / ST D/CAL should be within the ranges stated in the Quality Control Certificate of the lot.

The Calibrator and Controls are human sera, and as such they may show inhomogeneity, if their value in the test is significantly different from the values stated in the Certificate of analysis, consult the results with the manufacturer.

Precision

The interassay variability (between tests) and the intraassay variability (within the test) were determined by

testing samples with different OD values.

a. Repeatability (intraassay)

The variation coefficient of intraassay is max. 8 %. It is measured for each particular lot at least on 12 parallels of the same microtiter plate.

Example: (n = number of parallel wells on the same plate)

n	A	±σ	CV rep.
16	1.335	0.050	3.8 %
16	0.614	0.023	3.7 %

b. Reproducibility (interassay)

The variation coefficient of reproducibility is a maximum of 15 %. It is measured for each lot by comparing the wells of the same sample in several consecutive tests.

Example: (n = number of tests of a certain sample)

n	A	±σ	min – max	CVrepro
18	1.369	0.064	1.223 – 1.476	4.7 %
18	0.463	0.060	0.337 – 0.569	12.9 %

Detection Range

The measuring range is determined by the measuring capability of the spectrophotometer / colorimeter used.

Detection Limit

The limit of quantification is defined as the lowest measurable concentration that can be distinguished from zero with 95% confidence. This value is determined for each batch of the kit and is stated in the Quality Control Certificate of the given batch of the kit.

Sensitivity

Sensitivity and specificity of the test

The evaluation of the diagnostic sensitivity and the specificity of the test was performed by the comparing the TEST kit with two other commercial ELISA tests and with indirect immunofluorescence test.

Serum samples	Total	Positive	Equivocal	Negative	
Positive	73	68	4	1	Sensitivity: 98.6 %*
Negative	74	1	2	71	Specificity: 98.6 % *

* Equivocal results were not taken in account for calculation

a. Analytical sensitivity of the test

The analytical sensitivity of the assay is defined as the mean of the sample without analyte plus three times

of the standard deviation and represents the lowest detectable antibody titer. The analytical sensitivity value is determined for each kit lot and is stated in the Quality Control Certificate of that kit lot.

b. Analytical specificity of the test

The quality of the native antigen VZV, which recognizes specific antibodies in patient samples, ensures the high specificity and sensitivity of this assay. However, to determine the diagnosis, the test results must always be interpreted in the context of clinical signs and the results of other laboratory tests, see Interpretation Of Results).

Specificity

The quality of the antigen used ensures the high specificity and sensitivity of this kit. However, in order to detect potentially cross-reactive antibodies, the positive samples for Borrelia, TBEV, CMV, HHV-6, HSV, EBV, SARS-CoV-2, Mycoplasma pneumoniae, Chlamydia pneumoniae and Toxoplasma gondii were tested. No cross-reactivity with all tested samples was detected. However, in general, other cross-reactivities cannot be guaranteed.

Linearity

The quantification is accurate only in the linear part of the calibration curve, which represents the interval of OD values 0.100 – 2.400, in which the linear trend line satisfies the condition of reliability R to the second >0.95. If the measured OD of the sample exceeds this linearity interval, the test at the higher dilution must be repeated for accurate quantification.

Recovery

Measured values of recovery test for every Lot are between 80-120 % of expected value.

Interferences

Haemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of haemoglobin, 5 mg/mL of bilirubin and 50 mg/mL of triglycerides. Nevertheless, such samples can only be tested with reservations.

Precautions

1. All kit components are for laboratory use only.
2. The manufacturer guarantees the usability of the kit as a whole.
3. Wash buffer WASH 10×, Urea solution UREA, Chromogenic substrate TMB, Stop solution STOP, and Dilution buffer DIL are not interchangeable between kits.
4. Work aseptically to avoid microbial contamination of samples and reagents.
5. When collecting, diluting, and storing reagents, be careful not to cross-contaminate them or contaminate them with enzymatic activity inhibitors.
6. The Chromogenic Substrate TMB shouldn't come into contact with oxidizing agents and metal surfaces.

Because it is sensitive to light, close the bottle immediately after use. The Chromogenic substrate TMB must be clear in use. Do not use the solution if it is blue.

7. Follow the Instruction manual exactly. Non-reproducible results may arise in particular:

   insufficient mixing of reagents and samples before use

   inaccurate pipetting and non-compliance with the incubation times

   poor washing technique and splashing of the edges of the wells with sample or conjugate

   using the same tip when pipetting different solutions or swapping caps

8. Human control sera and standards used in the kit were tested for the absence of HBsAg, HCV and anti HIV-1,2 antibodies. Treat test specimens, control sera, standards, and used strips as infectious material. Autoclave items that have been in contact with them for 1 hour at 121°C or disinfect for at least 30 minutes with 3% chloramine solution.
9. Neutralize liquid waste containing Stop solution (sulfuric acid solution) with 4% sodium bicarbonate solution before disposal.
10. Disinfect the waste generated during strip washing in a waste container using a suitable disinfectant solution (eg Incidur, Incidin, chloramine, ...) at the concentration recommended by the manufacturer.
11. Handle Stop solution STOP carefully to avoid splashing on the skin or mucous membranes. If this happens, wash the affected area with plenty of running water.
12. Do not eat, drink or smoke while working. Do not pipette by mouth, but by suitable pipetting devices. Wear protective gloves and wash your hands thoroughly after work. Be careful not to spill specimens or form an aerosol.
13. All reagents and packaging material must be disposed of in accordance with applicable legislation.
14. In case of suspicion of an adverse event in connection with the use of the kit, inform the manufacturer and the competent state authority without delay.