ELISA-VIDITEST anti-JCV IgG

REF ODZ-450 Σ 96 tests 2°C 10°C 2°C - 10°C

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Type of determination: IgG antibodies

Type of evaluation: Qualitative, Semiquantitative, Quantitative

Type of samples: Serum/Plasma

Processing possibility: Manual

CE IVD

VIDIA

Instruction manual

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1. TITLE

ELISA-VIDITEST anti-JCV IgG

2. INTENTED USE

The kit is intended for professional use for the qualitative, semiquantitative and quantitative detection of IgG antibodies against polyomavirus JC (JCV) in human serum and plasma. The kit is intended for laboratory diagnostics of diseases caused by or associated with JCV (e.g. progressive multifocal leukoencephalitis (PML) in immunodeficient patients).

3. TEST PRINCIPLE

ELISA-VIDITEST anti-JCV IgG is a solid-phase immunoanalytical test. The surface of the wells is coated with recombinant species-specific JCV antigen. 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.

4. KIT COMPONENTS

ELISA break-away strips in the handling frame coated with the specific antigen STRIPS Ag	1 x 12 pcs
1.3 mL Negative control human serum, r.t.u. [1] NC	1 vial
1.3 mL Positive control human serum, r.t.u. PC	1 vial
2.0 mL Calibrator (human serum), r.t.u. CAL	1 vial
13 mL Anti-human IgG animal antibodies labelled with horseradish peroxidase (anti-IgG Px conjugate) r.t.u. CONJ	1 vial
55 mL Wash buffer, 10x concentrated WASH 10x	1 vial
60 mL Dilution buffer, r.t.u. DIL	1 vial
13 mL Chromogenic substrate TMB-O, r.t.u. TMB-O	1 vial
13 mL Stop solution, r.t.u. (0.4 M sulfuric acid) STOP	1 vial
Instruction manual	
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.

Chromogenic substrate TMB-O is compatible and interchangeable between ELISA-VIDITEST kits which contain TMB-O and not compatible with other Chromogenic substrates used in other ELISA-VIDITEST TMB, TMB-BF.

5. MATERIALS REQUIRED BUT NOT PROVIDED

Distilled/deionised water for dilution of the Wash buffer WASH 10x, pipetting equipment, equipment for liquid dispensing and strip washing, spectrophotometer/colorimeter.

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

6. REAGENTS PREPARATION

a. **Allow all kit components to reach room temperature.**

b. **Thoroughly mix Dilution buffer DIL, Conjugate anti-IgG Px CONJ and Chromogenic substrate TMB-O.**

c. **Thoroughly mix tested samples and control sera just prior to testing. Dilute the tested samples 101x** with Dilution buffer DIL **(e.g. 5 µL sample + 500 µL Dilution buffer DIL).**. **Do not dilute** control sera and calibrator, they are in working concentration (r.t.u., ready to use).

d. 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.

e. **Do not dilute** Conjugate anti-IgG Px CONJ, Chromogenic substrate TMB-O and Stop solution STOP, they are ready to use.

7. ASSAY PROCEDURE

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

a. 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.

b. Fill wells with 100 µL of control sera and diluted test samples as follows: Fill the first well with Dilution buffer DIL to determine the background of the reaction (BLANK). Fill two wells with Calibrator CAL, the next well with Positive control serum PC, the next well with Negative control serum NC, and the remaining wells with diluted test samples (S1, S2,…) (see Figure 1). Just apply each sample to one well. To rule out a possible laboratory error, apply CAL to three wells, test samples, and control sera in two wells. We recommend that a positive reference serum sample (internal control) be included in each test to verify the continuity and variability of the test.

   Incubate **60 minutes** (+/- 5 min) **at room temperature**.

c. 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.

d. Mix thoroughly the vial of anti-IgG Px conjugate CONJ and pipette 100 µL of anti-IgG Px conjugate CONJ into the wells.

   Incubate **60 minutes** (+/- 5 min) **at room temperature**.

e. Aspirate the fluid from the wells and wash them with 4 x 250 µL of wash solution. Aspirate and tap.

f. Pipette 100 µL of Chromogenic substrate TMB-O solution into the wells.

   Incubate for **10 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.

g. Stop the reaction by adding 100 µL of Stop solution STOP. Pipette at the same rate as the Chromogenic substrate TMB-O 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.

h. 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

	1	2	3	4	5	6	7	8	9	10	11	12
a	**DIL**	**S4**										
b	**CAL**	**S…**										
c	**CAL**											
d	**PC**											
e	**NC**											
f	**S1**											
g	**S2**											
h	**S3**											

8. 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.

8.1 Qualitative orientation evaluation

1. Calculate the mean OD value of the Calibrator CAL. If you are applying three calibrator 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 Calibrator 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.

8.2 Semiquantitative evaluation

Determine Positivity **Index** for each sample:

1. First determine the cut-off value as in the previous evaluation method (See paragraph 8.1, point 2).
2. Determine the index value for each sample by dividing the OD of the test sample by the cut-off value.
3. 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 Calibrator CAL	= 0.814; 0.876
Mean OD Calibrator CAL	= 0.845
OD sample	= 0.800
Correction factor Calibrator CAL	= 0.37
Cut-off value	= 0.845 x 0.37 = 0.313
Positivity index value	= 0.800 / 0.313 = 2.56

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.

8.3 Processing of results for Quantitative interpretation

Determination of the antibody concentration in the tested samples (AU/mL) using the E-CALCULATOR (www.vidia.cz).

In case of determination of the antibody amount in the units, use the E-CALCULATOR program, which calculates the **AU/mL** using a calibration curve constructed according to the current values of the Calibrator CAL and parameters A1, A2 and C. The OD values from the reader are entered into the program, depending on whether the drip scheme for one or two wells was used. For the correct calculation from the calibration curve, **it is necessary to specify parameter B / Bmax for Calibrator /CAL and then parameters A1, A2, C, gray zone range and Cmin and Cmax parameters** that are specific to each lot of the kit and are stated in **the Quality Control Certificate**.

The program evaluates the results automatically for the samples diluted 101x. The program automatically determines the concentration of antibodies in samples that were tested at a dilution of 101x. To calculate antibody concentrations (AU/mL) in samples that have been tested at another dilution, the program performs a recalculation after entering the current sample dilution in the appropriate column.

8.3.1. The evaluation of antibody concentration for sera/plasma (101x)

The evaluation in arbitrary units for sera/plasma 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 correct only in linear part of a calibration curve. If measured sample OD exceeds linearity range (indicatively it is in OD range 0,100 – 3,100; the exact range is stated in the Quality control certificate for the particular lot) it is necessary for correct quantification to repeat the test with more diluted sample and then to take into account this dilution during concentration calculations.

9. RESULT INTERPRETATION

50-60% of the population is infected with Polyoma JC virus in childhood. Infection occurs without symptoms and passes into a latent stage, which is associated with long-term presence of anamnestic IgG antibodies in the sample. In latently infected people, the virus may be reactivated repeatedly; they may be reinfected with another virus strain. Reactivation/reinfection can be associated with transient viremia or asymptomatic viral

shedding in urine. In rare cases in immunodeficient patients, reactivation or reinfection can cause infection of the central nervous system (PML). The development of this disease is influenced by many factors, both viral and host origin, and also by method of treatment. and for monitoring the of PML risk in patients treated with some types of immunomodulatory biological drugs, e.g., in patients receiving natalizumab. The recombinant antigen which is used in the test does not cross-react with other human polyomaviruses (Polyoma BK, polyomavirus Merkel cell carcinoma).

10. TEST CHARACTERISTICS

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

10.1 Validity of the test

The absorbance value of the Dilution buffer (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 PC / 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 (see CoA - lot characteristics), consult the results with the manufacturer.

10.2 Precision of the test

The interassay variability (between tests) and the intraassay variability (within the test) were determined by testing samples with different OD values.

10.2.1 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.
12	2.592	0.075	2.9 %

10.2.2 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
4	0.640	0.079	0.523 – 0.735	9.5 %
4	1.246	0.148	1.107 – 1.415	6.9 %
4	2.648	0.110	2.436 – 2.821	6.2 %

10.2.3 Recovery test

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

10.3 Diagnostic sensitivity and specificity of the test

The diagnostic sensitivity and specificity of the test was performed with a set of serum samples that were characterized by validated tests: a) reference ELISA test in National reference laboratory for papillomaviruses and polyomaviruses in Prague, b) ELISA STRATIFY™ JCV Dx SELECT test in reference laboratory in Copenhagen.

	Results with ELISA-VIDITEST anti-JCV IgG			
Results of comparative test	negative	equivocal	positive	sum
Seronegative	26	1	1	28
Seropositive	2	1	37	40

The diagnostic sensitivity of the test is 95 % and the specificity is 96 % (equivocal samples excluded)

10.4 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.

10.5 Analytical specificity of the test

The quality of the species-specific recombinant antigen JCV, which recognizes specific antibodies in patient samples, ensures the high specificity and sensitivity of this assay. The recombinant antigen which is used in the test does not cross-react with other human polyomaviruses (Polyoma BK, polyomavirus Merkel cell carcinoma).

10.6 Measuring range

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

10.7 Linearity

The quantification is accurate only in the linear part of the calibration curve, in which the linear trend line satisfies the condition of reliability $R^2 > 0.95$. Indicatively it is in OD range 0,100 – 3,100; the exact range is stated in the Quality control certificate for the particular lot. If the measured OD of the sample exceeds this linearity interval, the test at the higher dilution must be repeated for accurate quantification.

10.8 Interference

Haemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of haemoglobin, 10 mg/mL of bilirubin and 50 mg/mL of triglycerides. Nevertheless, such samples can only be tested with reservations.

10.9 Limit of quantification

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.

11. WARNINGS

a. All kit components are for laboratory use only.

b. The manufacturer guarantees the usability of the kit as a whole.

c. Wash buffer WASH 10x, Chromogenic substrate TMB-O, Stop solution STOP, and Dilution buffer DIL are interchangeable between ELISA-VIDITEST kits, unless otherwise noted in the kit instructions.

d. Work aseptically to avoid microbial contamination of samples and reagents.

e. When collecting, diluting, and storing reagents, be careful not to cross-contaminate them or contaminate them with enzymatic activity inhibitors.

f. The Chromogenic Substrate TMB-O 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-O must be clear in use. Do not use the solution if it is blue.

g. 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 given in Chapter 7
   - 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

h. 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.

i. Neutralize liquid waste containing Stop solution (sulfuric acid solution) with 4% sodium bicarbonate solution before disposal.

j. 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.

k. 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.

l. 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.

m. All reagents and packaging material must be disposed of in accordance with applicable legislation.

n. 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.

12. SAFETY PRECAUTIONS

Calibrator CAL, Control Sera PC and NC and Dilution buffer DIL are preserved with ProClin 300 (a mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1)). Therefore, the following warnings and safety precautions apply to these solutions:

Warning	H317	May cause an allergic skin reaction.
	H411	Toxic to aquatic life with long lasting effects.
	P280	Wear protective gloves/protective clothing/ protective glasses/ face protection.
	P302+P352	OF ON SKIN: Wash with plenty of water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before reuse.

The anti-IgG Px conjugate CONJ contains N-methyl-2-pyrrolidone. Therefore, the following warnings and precautions apply to this solution:

Danger	H360D	Warning: May damage the unborn child.
	P202	Do not use until you have read and understood all safety instructions.
	P280	Wear protective gloves/protective clothing/ protective glasses/ face protection.
	P308+P313	If exposed or concerned: Get medical advice/attention.
	P501	Dispose of contents/container in accordance with local regulations.

Further information can be found in the safety data sheet.

13. STORAGE AND EXPIRATION

It is recommended to use the kit within three months after opening.

a. Store the kit and the kit reagents at +2 °C to +10 °C, in a dry place and protected from the light. Under these conditions, the expiration period of the entire kit is indicated on the central label on the kit package, the expiration date of the individual components is indicated on their package.

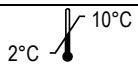
b. Put unused strips back in the package and seal or close tightly in a zippered bag with desiccant.

c. The kits are transported refrigerated in thermal bags, transport time up to 72 hours has no influence on expiration. If, upon receipt of the kit, you notice serious damage to the packaging of any component of the kit, inform the manufacturer immediately.

d. Store unused test samples undiluted, aliquoted and frozen at -18 °C to -28 °C. Frequent freezing and thawing is not recommended. If you store samples at + 2 °C to + 10 °C, then test them within one week.

e. Test sample solutions at the working concentration cannot be stored. Always prepare them fresh.

14. USED SYMBOLS

Symbol	Explanation
	number of tests
CE	Conformité Européenne – product meets the requirements of European legislation
IVD	diagnostics *in vitro*
±σ	standard deviation
CV	coefficient of variation
OD	optical density
	manufacturer
	expiration
LOT	lot of kit
	storage at +2 °C - +10 °C
°C	Celsius degree
%	percentage
n	number of tested samples
A	value of a certain sample
	read the package leaflet
REF	catalog number

15. TEST SCHEME

Step 1. Prepare reagents and test samples in working concentration

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Step 2. Apply 100 μL/well of control sera and test samples

↓

Incubate 60 minutes at room temperature

↓

Wash 4 times (250 μL/well), aspirate

↓

Step 3. Apply 100 μL/well of anti-IgG Px conjugate

↓

Incubate 60 minutes at room temperature

↓

Wash 4 times (250 μL/well), aspirate

↓

Step 4. Apply 100 μL/well of Chromogenic substrate TMB-O

↓

Incubate 10 minutes in dark at room temperature

↓

Step 5. Apply 100 μL/well of Stop solution

↓

Step 6. Read the absorbance at 450 / 620-690 nm within 10 minutes

References:

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Kean J.M., Rao S., Wang M., Garcea R.L.: Seroepidemiology of human polyomaviruses. PloS Pathogens 5: e1000363, 1-10, 2003.

Stolt A.,Sasnauskas K., Koskela P., Lehtinen M., Dillner J.: Seroepidemiology of human polyomaviruses. J.Gen.Virol. 84: 1499-1504, 2003.

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