ELISA-VIDITEST anti-BKV IgG

REF RDZ-405 Σ 96 tests 2°C 10°C 2°- 10 °C

Type of determination: IgG antibodies

Type of evaluation: Qualitative, Semiquantitative

Type samples: Serum/Plasma

Processing: Manual

ELISA-VIDITEST anti-BKV IgG

RDZ-405

Instruction manual

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1. TITLE

ELISA-VIDITEST anti-BKV IgG – ELISA kit for the detection of species-specific IgG antibodies against polyomavirus BK (BKV) in human serum and plasma.

2. INTENDED USE

The kits can be used for detection of anti BKV IgG antibodies in human serum or plasma samples.

3. TEST PRINCIPLE

ELISA-VIDITEST anti-BKV IgG is a solid-phase immunoanalytical test. The surface of the wells is coated with recombinant species-specific BKV antigen. If present in the serum samples, respective antibodies bind to the immobilized antigen. In the next step, bound antibodies react with anti-human IgG antibodies labeled with horseradish peroxidase. The amount of bound labeled antibodies is determined by colorimetric enzymatic reaction. Negative sera do not react and the mild change in color, if present, may be attributed to the reaction background.

4. KIT COMPONENTS

ELISA break-away strips coated with specific antigens	STRIPS Ag	1 x 12 pcs
1.3 mL	Negative control serum, r.t.u.[1)] NC	1 vial
1.3 mL	Calibrator, r.t.u. CAL	1 vial
1.3 mL	Positive control serum, r.t.u. PC	1 vial
13 mL	Anti-human IgG antibodies labelled with horseradish peroxidase (anti-IgG Px-conjugate), r.t.u. CONJ	1 vial
55 mL	Wash buffer concentrate, 10x concentrated WASH 10x	1 vial
60 mL	Dilution buffer, r.t.u. DIL	1 vial
13 mL	Chromogenic substrate (TMB-O substrate), r.t.u. TMB-O	1 vial
13 mL	Stop solution, r.t.u. 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 r.t.u. is only compatible and interchangeable between ELISA-VIDITEST kits which contain TMB-O and IT IS NOT INTERCHANGEABLE with other TMB substrates TMB, TMB-BF used in other ELISA-VIDITEST kits.

5. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Distilled or deionised water for diluting of the Wash buffer concentrate, appropriate equipment for pipetting, liquid dispensing and washing, spectrophotometer/colorimeter/microplate reader – wavelength 450 nm (620-690 nm reference filter recommended, not required).

6. PREPARATION OF REAGENTS AND SAMPLES

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

b. **Prior to use mix well serum samples, standards and conjugate in order to ensure homogeneity.** Dilute serum samples **101 times in Dilution buffer (e.g. 5 µL of serum sample + 500 µL of Dilution buffer).** Do not dilute the Standards, they are ready to use.

Note: If you examine more than 30 serum samples at one time, it is recommended to perform the dilution in a microtiter plate and transfer the samples into the test plate with a multichannel pipette. This procedure will allow to reach the desired incubation time for all sera.

c. Prepare Wash buffer by diluting the Wash buffer concentrate 10 times in an appropriate volume of distilled or deionised water (e.g. 50 mL of the concentrated Wash buffer + 450 mL of distilled water). If crystals of salt are present in the concentrated Wash buffer, warm up the vial to +32 to +37°C in a water bath. Diluted Wash buffer is stable for one month if stored at room temperature.

d. **Do not dilute** Px-conjugate, TMB-O substrate and Stop solution, they are ready to use.

7. ASSAY PROCEDURE

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

a. Allow the microwell strips sealed inside the aluminum bag to reach room temperature in order to prevent condensation on the strips. Withdraw an adequate number of strips and put the unused strips into the provided pouch and seal it carefully with the desiccant kept inside.

b. Fill the wells with 100 µL of Standards and diluted serum samples in the following manner: Start with filling the first well with 100 µL of negative control serum NC. Fill two wells with 100 µL/well of Calibrator CAL, next well with Positive control serum PC and then pipette the diluted serum samples (S1, S2,…) (see Tab. 1). It is sufficient to apply the serum samples in one well. If you wish to minimize the laboratory error apply the Calibrator in three wells and the samples and control sera in two wells. We recommend including a 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 room temperature.

c. Aspirate the liquid from wells into a waste bottle containing an appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 250 µL/well of Wash buffer. Avoid cross-contamination between wells. If any liquid stays trapped inside the wells, invert the plate and tap it on an adsorbent paper to remove the remaining drops.

d. Mix well the bottle with anti-IgG Px-conjugate CONJ and add 100 µL of anti-IgG Px-conjugate r.t.u. into each well. **Incubate 30 minutes (±2 min)** at room temperature.

e. Aspirate and wash four times with 250 µL/well of Wash buffer. (see c.)

f. Dispense 100 µL of TMB-O substrate into each well. Incubate for **10 minutes** (+/-30 seconds) at room temperature in dark. **The time measurement must be started at the beginning of TMB-O dispensing.** Follow this rule and keep the time interval. Pipette fast in regular rhythm or use appropriate dispensing device. Cover the strips with an aluminum foil or keep them in the dark during the incubation with TMB-O substrate.

g. Stop the reaction by adding 100 µL of Stop solution STOP. Use the same pipetting rhythm as with the TMB-O substrate to ensure the same reaction time in all wells. Check that no air-bubbles are present inside the well otherwise tap gently the microplate to remove them.

h. Measure the absorbance at 450 nm with a microplate reader **within 10 minutes** after termination of the reaction. It is recommended to use a reference reading at 620-690 nm.

Table 1: Pipetting scheme for semiquantitative analysis

	1	2	3	4	5	6	7	8	9	10	11	12
a	**NC**	**S5**										
b	**CAL**	**S…**										
c	**CAL**											
d	**PC**											
e	**S1**											
f	**S2**											
g	**S3**											
h	**S4**											

8. TEST EVALUATION

8.1. Qualitative interpretation

1. Compute the absorbance mean of Calibrator CAL. If you applied calibrator into 3 wells and if any of the three values falls out of the range more than 20% of the mean absorbance then exclude the deviating well from the calculation and compute a the mean using the values from the other two wells.
2. Compute the cut-off value by multiplying the OD Calibrator mean with a Correction factor. **The Correction factor value for the particular Lot is written in enclosed Quality control certificate.**
3. Samples with absorbance lower than 90% of the cut-off value are considered negative and samples with absorbance higher than 110% of the cut-off value are considered positive. The samples with absorbance in the range of 90-110% of cut-off value are equivocal (see note in par. 8.2.)

8.2. Semiquantitative interpretation

1. Determine the cut-off value as previously in 8.1. (2.)
2. Compute the Index value for each serum sample by dividing the test serum absorbance by cut-off value.
3. Interpret the results according to the following table (Results interpretation):

RESULTS INTERPRETATION

Index value	Interpretation
< 0.90	Negative
0.90 - 1.10	+/-
> 1.00	Positive*

*****based on the value of antibody index, it is possible to semiquantitatively estimate the amount of antibodies in samples

Example of calculation:

Standard D absorbances	= 0.814; 0.876
Mean of Standard D	= 0.845
Sample absorbance	= 0.800
Correction factor of Standard D	= 0.37
Cut-off value	= 0.845 x 0.37 = 0.313
Index value	= 0.800 / 0.313 = 2.56

Note! An indifferent sample reactivity, i.e. interpreted as +/-, requires repetition of the sample testing. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain another sample from the patient.

9. RESULTS INTERPRETATION

50-80% of the population is infected with Polyoma BK 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 serum. BKV occurs in four genotypes, characterized by a different sequence of major neutralizing antigenic determinants on the major capsid protein VP1. Representation of individual genotypes in the general population is regionally different: genotype I prevails worldwide (47-82%), followed by genotype IV (5-54%). Genotypes II - III are rarer (0-9%). In latently infected people, the virus may be reactivated repeatedly or they may be reinfected with another virus strain. Reactivation/reinfection can be associated with transient viremia or asymptomatic viral shedding in urine. In immunodeficient patients, reactivation or reinfection may cause diseases of the urogenital tract, in rare cases even a generalized infection associated with various types of organ disorder. A high risk of complications is associated primarily with primary infection of the patient in a state of immunosuppression, so it is appropriate to know the serostatus of the graft donor in patients with transplant, particularly in recipients of the kidney transplant. The recombinant antigen used in the assay includes two of the most frequently represented genotypes of BKV (type I and IV) and does not cross-react with other human polyomaviruses (polyoma JC, Merkel cell polyomavirus). However, it can cross-react with monkey polyomavirus SV40.

10. CHARACTERISTICS OF THE TEST

10.1 Validity of the test

The mean absorbance values of standards/control sera and the ratio between the absorbance values of PC / CAL should be in the ranges stated in the **Quality control certificate** for this kit lot.

The test is designed to determine specific IgG antibodies in human serum and plasma.

10.2 Precision of the test

The intra-assay variability (within the test) and the inter-assay reproducibility (between tests) were determined using samples with different absorbance values.

10.2.1. Variability (intra-assay)

The coefficient of intraassay variability is max. 8%. It is measured for each particular Lot as absorbance of minimum 12 parallel wells for the particular microtitrate plate.

Example:

(n= number of parallel determinations in the same test)

n	A	±σ	CV
16	1.893	0.032	2.6 %

10.2.2. Reproducibility (inter-assay)

The coefficient of interassay variability is max. 15%. It is measured for each particular Lot as comparison of the absorbances of the same serum sample in several consecutive tests.

Example:

(n= number of determinations of the same sample)

n	A	±σ	CV
6	0.341	0.043	12.6%
6	1.525	0.128	8.4 %
6	2.24	0.088	3.9%

10.3 Diagnostic sensitivity and specificity of the test

Since there is no commercially available kit for the determination of type-specific antibodies against BKV, a test of the diagnostic efficiency was performed using serum or plasma of defined donors (recipients of kidney transplant, blood donors, children aged 1 year) and on the basis of comparative testing of a panel of serum delivered by the National Reference Laboratory for Papillomaviruses and Polyomaviruses. In total, 222 serum or plasma samples were examined. The prevalence of antibodies found in each serum/plasma group was consistent with the assumption and with the published data: a high antibody prevalence (83 and 87%) was detected in the plasma group from the Tx kidney recipients and in the serum group from healthy blood donors, similar to seroprevalence studies by Keana et al., 2009, or Stolt et al., 2003. IgG antibodies against BKV were detected in all 10 Tx kidney recipients and were detected by PCR for active BKV infection. In children aged 1 year, who had previously not been infected with BKV and had already decreased maternal antibody levels, seroprevalence of 5% was found. In a comparative test of 50 samples that were tested by the reference method in the NRL, a 93% agreement was found.

These results show that ELISA-VIDITEST against BKV IgG has sufficient diagnostic sensitivity and specificity.

10.4. Recovery test

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

10.5. Interference

Hemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of hemoglobin, 5 mg/mL of bilirubin and 50 mg/mL of triglycerides.

11. SAFETY PRECAUTIONS

All ingredients of the kit are intended for laboratory use only.

Standards contain human sera that has been tested negative for HBsAg, anti-HIV-1,2 and anti-HCV. However they should be regarded as contagious and handled and disposed of according to the appropriate regulations.

Autoclave all reusable materials that were in contact with human samples for 1 hour at 121°C, burn disposable ignitable materials, decontaminate liquid wastes and nonignitable materials with 3% chloramine. Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution. The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g. Incidur, Incidin, chloramin,...) in concentrations recommended by the producer.

Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.

Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.

12. HANDLING PRECAUTIONS

a. Manufacturer guarantees performance of the entire ELISA kit.

b. Wash buffer, Chromogenic substrate TMB-O, Stop solution and Dilution buffer are interchangeable between ELISA-VIDITEST kits unless otherwise stated in the instruction manual.

c. Avoid microbial contamination of serum samples and kit reagents.

d. Avoid cross-contamination of reagents when handling, diluting and storing reagents.

e. Calibrator and control sera contain preservative ProClin 300® (mix of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-iothiazol-3-one (3:1)).

f. Avoid contact of the TMB-O substrate with oxidizing agents or metal surfaces.

g. Follow the assay procedure indicated in the Instruction manual. Variations in the test results are usually due to:

- Insufficient mixing of reagents and samples
- Inaccurate pipetting and inadequate incubation times
- Poor washing technique or spilling the rim of well with sample or Px-conjugate

- Use of identical pipette tip for different solutions

13. STORAGE AND EXPIRATION

The ELISA kit should be used within three months after opening.

a. Store the kit and the kit reagents at +2 to +10°C, in a dry place and protected from the light. Under these conditions, the expiration date is indicated at the ELISA kit label and at all reagent labels.

b. Kits are shipped in cooling bags, the transport time of 72 hours have no influence on expiration. If you find damage at any part of the kit, please inform the manufacturer immediately.

c. Store unused strips in the sealable pouch and keep the desiccant inside.

d. Store undiluted serum samples at +2 to +10°C up to one week. For longer period make aliquots and keep them at -18 to -28°C. Avoid repeated thawing and freezing.

e. Solutions of diluted serum samples at working concentration cannot be stored. They need to be prepared fresh.

14. USED SYMBOLS


number of tests

±σ standard deviation

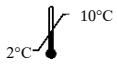
CV coefficient of variation

OD optical density


manufacturer


expiration

Lot of the kit



storage at +2°C - +10°C

°C Celsius degree

% percentage

n number of tested samples

A value of tested sample


read usage instructions

catalog number

15. RECOMMENDED LITERATURE:

Šroller V.,Hamšíková E.,Ludvíková V.,Vochozková P., Kojzarová M., Fraiberg M., Saláková M., Morávková A.,Forstová J., Němečková Š.: Seroprevalence rates of BKV, BKV and MCPyV polyomaviruses in the general czech population. J.Med. Virol.86: 1560-1568, 2014.

Viscidi R.P.,Rillison D.E.,Sondak V.K. et al.: Age-specific seroprevalence of Merkel Cell Polyomavirus, BK virus and JC virus. Clin. Vaccine Immunol. 18:1737-1743, 2011.

Kean J.M., Rao S., Wang M., Garcea R.L.: Seroepidemiology of human polyomaviruses. PloS Pathogens 5: e1000363, 1-10, 2009.

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

Pastrana D.,Ray U.,Magaldi T.G. et al.:BK polyomavirus genotypes represent distinct serotypes with distinct entry tropism. J.Virol. 87:10105-10113, 2013.

Pastrana D., Brennan D.C., Cuburu N. et al.: Neutralization serotyping of BK polyomavirus infection in kidney transplant recipients. PLoS pathogens 8, 2012, e1002650.

Krumbholz A.,Zell R.,Egerer R., et al.: Prevalence of BK virus genotype I in Germany. J.Med.Virol. 78: 1588-1598, 2006.
Rinaldo C.H., Tylden G.D., Sharma B.N.: The human polyomavirus BK: virological background and clinical implications. Acta Pathol. Immunol.Scand. 121:728-745,2013.

15. FLOW CHART

Step 1	**Prepare reagents and samples at working concentrations**
	↓
Step 2	**Dispense 100 µL/well of standards and samples**
	↓
	Incubate 30 minutes at room temperature
	↓
	Wash 4 times (250 µL/well), aspirate
	↓
Step 3	**Dispense 100 µL/well of Px-conjugate r.t.u.**
	↓
	Incubate 30 minutes at room temperature
	↓
	Wash 4 times (250 µL/well), aspirate
	↓
Step 4	**Dispense 100 µL/well of TMB-O substrate**
	↓
	Incubate 10 minutes at room temperature
	↓
Step 5	**Dispense 100 µL/well of Stop solution**
	↓
Step 6	**Read the absorbance at 450 / 620-690 nm within 10 minutes**

The kit was developed within the project TH-01010548 with the financial support of the Technology Agency of the Czech Republic.

Date of the last revision of this manual: 04/2021