EBV VCA-IgG-ELISA PKS medac

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EBV VCA-IgG-ELISA PKS medac

Enzyme immunoassay with Pipetting Control System for the quantitative detection of IgG antibodies to Epstein-Barr virus-specific capsid antigen (VCA) in serum

Cat. no.: 128-PKS

FOR IN VITRO DIAGNOSTIC USE ONLY

INTRODUCTION

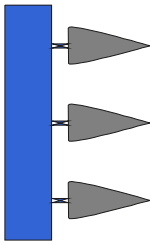
Epstein-Barr virus (EBV) belongs to the family of *Herpesviridae*. It consists of a double-stranded DNA genome, capsid, tegument and envelope. It is characteristic for EBV to persist latently and livelong in the organism after primary infection.

90 to 95 % of all adult individuals are infected with EBV worldwide. The course, EBV primary infections take in immunocompetent individuals in early childhood, is normally without any symptoms. In young adults an EBV infection clinically manifests as glandular fever (Pfeiffer`s disease). In individuals who suffer from minor immunosuppression a clinically silent reactivation of the virus may occur. In individuals who suffer from massive immunosuppression EBV associated polyclonal B-cell lymphomas are observed. In 60 % of all Hodgkin lymphomas EBV DNA is present. Endemically relevant EBV-associated tumor disease are Burkitt`s lymphoma and nasopharyngeal carcinoma.

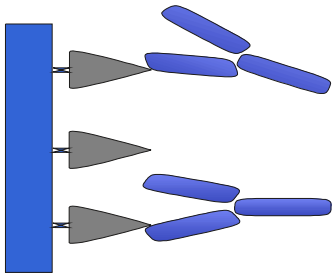
Relevant antigen complexes for antibody detection are the EBV-specific nuclear antigen (EBNA), the virus capsid antigen (VCA) and the early antigen (EA). In immunocompetent individuals antibody detection for distinction of primary infection towards previous infection or seronegativity is the focus of EBV diagnosis. EBV serology may be of importance as additional diagnostic of lymphadenopathy and of unclear neurological disease. In the context with a relevant EBV serology EBNA-1 IgG determination is the first step. A positive result confirms a previous infection. In the case of a negative EBNA-1 IgG, the determination of VCA IgG and IgM is essential to differentiate the status of infection.

The EBV VCA-IgG-ELISA PKS medac detects IgG antibodies specifically directed against the virus capsid protein gp 125. The test can be carried out fast and easily. By using a calibration curve it is possible to quantify the antibody concentration allowing a reliable follow-up.

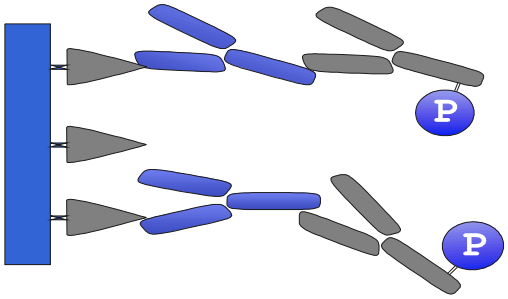
TEST PRINCIPLE



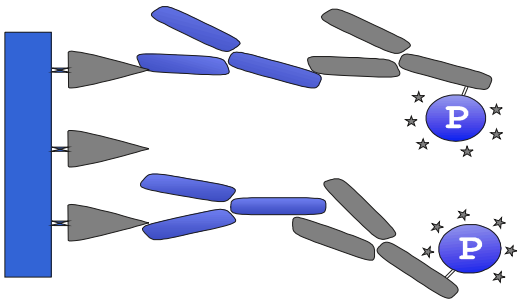
The plate is coated with EBV VCA gp 125 virus antigen.



The VCA gp 125-specific anti-bodies from the specimen are selectively bound to the antigen.



Peroxidase-conjugated anti-human IgG antibodies bind to the VCA gp 125-specific IgG antibodies (P = peroxidase).



Incubation with TMB-substrate (*). The reaction is stopped by the addition of sulfuric acid. The absorption is read photo-metrically.

Advantages of the test

- ☞ The Pipetting Control System allows to monitor visually each pipetting step through colour changes.
- ☞ The breakable microwell strips permit efficient use of the test.
- ☞ Suitable for automation on open ELISA devices.
- ☞ Single-point quantification, no standard curve needed.

KIT CONTENTS
Cat. no.: 128-PKS

1. **MTP**
   Microplate: 12 x 8 wells, green-coded (with frame and desiccant vacuum sealed in aluminium bag), breakable, U-form, coated with purified EBV VCA gp 125 antigen, BSA and pH indicator, ready to use.

2. **CONTROL** **-**
   Negative control: 1 vial with 1.5 ml, human serum, ready to use, contains BSA, phenol, ProClin™ 300 and gentamycin sulfate.

3. **CONTROL** **+**
   Positive control: 1 vial with 1.5 ml, human serum, ready to use, contains FCS, BSA, phenol, ProClin™ 300 and gentamycin sulfate.

4. **CAL**
   Calibrator: 1 vial with 1.5 ml, human serum, ready to use, contains FCS, BSA, phenol, ProClin™ 300 and gentamycin sulfate.

5. **WB**
   Wash buffer: 1 bottle with 100 ml, PBS/Tween (10x), pH 7.2 - 7.4, contains ProClin™ 300.

6. **VIR-DIL**
   Sample diluent: 1 bottle with 110 ml, PBS/Tween/BSA, pH 7.2 - 7.4, ready to use, contains ProClin™ 300.

7. **CON**
   Conjugate: 3 vials with 4.5 ml each, goat anti-human IgG, HRP-conjugated, ready to use, stained green, contains BSA, phenol, ProClin™ 300 and gentamycin sulfate.

8. **TMB**
   TMB-substrate: 1 vial with 10 ml, ready to use.

9. **STOP**
   Stop solution: 2 vials with 11 ml each, 0.5 M sulfuric acid (H_2SO_4), ready to use.

1. STORAGE AND STABILITY

Material/Reagent	State	Storage	Stability
Test kit	unopened	2...8 °C	until expiry date
Microplate	opened	2...8 °C in bag with desiccant	6 weeks
Controls/ Calibrator	opened	2...8 °C	6 weeks
Wash buffer	diluted	2...8 °C	6 weeks
Sample diluent	opened	2...8 °C	6 weeks
Conjugate	opened	2...8 °C	6 weeks
TMB-substrate	opened	2...8 °C	6 weeks
Stop solution	opened	2...8 °C	until expiry date

Do not use the reagents after the expiry date.

2. REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

2.1. Water for injection (H_2O redist.). Use of deionised water can disturb the test procedure.

2.2. Adjustable micropipettes.

2.3. Clean glass or plastic containers for dilution of wash buffer and specimen.

2.4. Suitable device for microplate washing (e.g. multistepper or ELISA washer).

2.5. Incubator for 37 °C.

2.6. Microplate reader with filters for 450 nm and 620 - 650 nm.

3. PREPARATION OF THE REAGENTS

Before starting the test procedure all kit components must be equilibrated to room temperature.

Calculate the number of wells required.

3.1. Microplate

The aluminium bag has to be tightly resealed together with the desiccant after each removal of wells. Storage and stability of the wells are indicated under point 1.

Note: The microplate wells have a light green colour. Possibly occurring greenish brown stains inside the wells are due to the production process and do not influence the test performance.

3.2. Wash buffer

Mix one volume of wash buffer (10x) with nine volumes of water for injection (e.g. 50 ml wash buffer (10x) with 450 ml water). 10 ml of diluted wash buffer are needed for eight wells.

Crystals in the wash buffer (10x) have to be dissolved by warming (max. 37 °C) and/or stirring at RT.

Do not mix test specific reagents (microplate, controls, conjugate, calibrator) from different kit lots. In contrast to that sample diluent, wash buffer, TMB-substrate and stop solution are generally exchangeable in all virological ELISA medac.

Reagents from other manufacturers must not be used in general.

Valid and reproducible results are only obtained if the test procedure is precisely followed.

4. SPECIMEN

4.1. The test is suitable for serum.

4.2. Pretreatment of sera, e.g. inactivation, is not necessary. However, they should neither be contaminated with microorganisms nor contain any red blood cells.

4.3. Sera have to be diluted 1:200 with sample diluent. We recommend to prepare an initial dilution of 1:50 (e.g. 10 µl serum + 490 µl sample diluent). For further 1:4 dilution just prepare the volume needed. Samples outside the measuring range can be diluted further.

5.A. TEST PROCEDURE

5.1. Cut the aluminium bag above the zip fastener and take out the required number of microplate wells (see 3.1.).

Microplate wells are ready to use and do not have to be pre-washed.

5.2. Leave well A1 empty as blank (see 6.A.). Add 50 µl each of the negative control, the positive control as well as the diluted samples in single determination, and 50 µl of the calibrator in duplicate to the wells.

After pipetting the samples (pH neutral or basic fluid) the wells turn blue/green. A missing colour change in one well indicates that no sample or control has been added.

If necessary, the microplate wells can be kept in a humid chamber up to 30 min at RT before proceeding.

5.3. Incubate the microplate wells for 60 min (± 5 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil.

5.4. After incubation wash the microplate wells three times with 200 µl wash buffer per well. Pay attention that all wells are filled. After washing tap microplate wells on filter paper.

Do not allow the wells to dry out! Proceed immediately!

5.5. Add conjugate (coloured green) to each well (except A1).

50 µl of conjugate have to be pipetted into the wells if the test is done manually.

Please note:
When working with automated devices, 60 µl of conjugate have to be pipetted into each well due to a higher evaporation in the incubation chambers of the devices.

The suitability of the test for automated devices was confirmed during the evaluation of the test. Nevertheless we recommend to verify the compatibility of the test with the devices used in the lab.

5.6. Incubate again for 60 min (± 5 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil.

5.7. After incubation wash microplate wells again (see 5.4.).

5.8. Add 50 µl of TMB-substrate to each well (also A1) and incubate for 30 min (± 2 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil in the dark. Positive samples turn blue.

5.9. Stop the reaction by adding 100 µl of stop solution to each well (also A1). Positive samples turn yellow.

Clean microplate wells from underneath before the photometric reading and take care that there are no air bubbles in the wells.

The reading should be done within 15 min after adding the stop solution.

5.B. TABLE FOR THE TEST PROCEDURE

	Blank (A1)	Negative control	Positive control	Calibrator	Sample
Neg. control	-	50 µl	-	-	-
Pos. control	-	-	50 µl	-	-
Calibrator	-	-	-	50 µl	-
Sample	-	-	-	-	50 µl
Incubate for 60 min at 37 °C, wash 3 x with 200 µl wash buffer					
Conjugate	-	50/60 µl*)	50/60 µl*)	50/60 µl*)	50/60 µl*)
Incubate for 60 min at 37 °C, wash 3 x with 200 µl wash buffer					
TMB-substrate	50 µl	50 µl	50 µl	50 µl	50 µl
Incubate for 30 min at 37 °C in the dark					
Stop solution	100 µl	100 µl	100 µl	100 µl	100 µl
Photometric reading at 450 nm (ref. 620 - 650 nm)					

*) manual/automatic procedure (see 5.5.)

6.A. CALCULATION OF RESULTS (VALIDITY)

* Read OD values at 450 nm (reference wavelength 620 - 650 nm).

* Subtract the OD value of the blank (well A1) from all other OD values.

* Lot-specific data
  The lot-specific data sheet provided with the kit contains the following information:
  - Lot-specific calibration curve
  - Curve parameters a and b
  - Nominal OD value of the calibrator
  - Lower OD limit of the calibrator
  - Nominal concentration range (AU/ml) of the positive control

* Validity criteria
  - The OD value of the **negative control** has to be **< 0.150**.
  - The unit value of the **positive control** has to be within the nominal range indicated in the lot-specific data sheet.
  - The mean OD value of the **calibrator** has to be above the lower OD limit indicated in the lot-specific data sheet.

Repeat the run if the results do not meet the specification.

* Correction of the results
The measured OD value of the positive control and the samples have to be corrected as follows:

$$OD_{corrected} = \frac{\text{Nominal OD value of the calibrator}}{\text{Measured OD value of the calibrator}} \times OD_{measured}$$

* Quantification of the results
The corresponding concentrations of the corrected OD values in AU/ml can be read from the lot-specific calibration curve (see lot-specific data sheet).

Alternatively, the concentrations can be calculated using the following formula:

$$\text{Concentration [AU/ml]} = b/\left(\frac{a}{OD_{corrected}} - 1\right)$$

Most of the new ELISA readers allow to program the formula, thus enabeling an automated data processing.

The measuring range spans from 9 to 200 AU/ml. Samples below this range have to be interpreted as < 9 AU/ml, those above as > 200 AU/ml. These values must not be extrapolated.

The cut-off is 10 AU/ml.

Grey zone = cut-off ± 10 % (= 9 to 11 AU/ml)

Attention! Important Note!

Due to the mathematical algorithm of the quantification negative or not defined AU values can be obtained in the following cases:

- Highly positive samples with corrected OD-values ≥ a are calculated as negative or not defined AU values (not allowed division by 0). These samples have to be retested in higher dilutions or have to be interpreted as > 200 AU/ml.

6.B. INTERPRETATION OF RESULTS/LIMITATIONS OF THE METHOD

* Samples with Unit values below the lower limit of the grey zone are reported as **NEGATIVE**.

* Samples with Unit values within the grey zone are reported as **EQUIVOCAL**. These samples should be retested together with a fresh specimen taken 14 days later in order to determine a titer change.

* Samples with Unit values exceeding the upper limit of the grey zone are reported as **POSITIVE**.

* The results should always be interpreted in connection with clinical data of the patients as well as the EBNA-1 IgG, the VCA IgM results and additional diagnostic parameters.

* Cross-reactions, caused by antibodies against other herpes viruses, cannot be excluded in single cases.

* Increased concentrations of lipids and of hemoglobin in samples do not influence the test results.

6.C. DIAGNOSTIC INTERPRETATION OF EBV SEROLOGY WITH MEDAC ASSAYS

The following interpretation of test results regarding the status of infection is only valid for the investigation of immuno-competent individuals.

EBNA-1 IgG	VCA IgG	VCA IgM	Interpretation
-	-	-	seronegative
+	+/±/-	-	past infection
-	+/±/-	+	primary infection

All other constellations have to be interpreted as unclear. In the event of an unclear result extended differential diagnostic methods would be necessary for interpretation.

In order to reduce the number of ambiguous constellations we recommend the application of medac’s EBV ELISA panel.

7. PERFORMANCE CHARACTERISTICS

We determined the following performance characteristics during the diagnostic evaluation.

7.A. SENSITIVITY AND SPECIFICITY

352 sera were measured during the diagnostic evaluation. The results were correlated with the diagnostical characterization of the lab Prof. Dr. Enders and colleagues, Stuttgart and the Institute of Virology of the University Homburg/Saar. The results are shown in the table below:

		Pre-definition (IFA)		
		negative	cut-off	positive
EBV VCA-IgG-ELISA PKS medac	negative	**184**	**0**	**5**
	cut-off	**0**	**0**	**1**
	positive	**4**	**0**	**158**

Sensitivity = 96.3 %
Specificity = 97.9 %

Concordance: 97.2 %

7.B. PRECISION

Sample	**Intra-assay variation**				**Sample**	**Inter-assay variation**			
	mean AU	**SD**	**CV (%)**	**n**		**mean AU**	**SD**	**CV (%)**	**n**
PC	36.1	1.52	4	23	**PC**	36.0	1.03	3	13
N° 1	8.0	0.47	6	23	**N° 6**	7.9	0.24	3	13
N° 2	28.1	1.04	4	22	**N° 7**	16.5	0.72	4	13
N° 3	28.9	2.73	9	22	**N° 8**	25.4	4.72	19*)	13
N° 4	69.1	3.10	5	22	**N° 9**	26.7	1.07	4	13
N° 5	112.1	8.87	8	22	**N° 10**	108.1	5.46	5	13
					N° 11	122.8	9.50	8	13

PC = positive control; *)acute serum (VCA IgM positive)

GENERAL HANDLING ADVICES

* To avoid cross contamination do not exchange the vials and their screw caps.

* The reagents have to be sealed immediately after use to avoid evaporation and microbial contamination.

* After use, the reagents have to be stored as indicated to guarantee the shelf life.

* After use, all components of the testkit should be stored in the original package, in order to avoid mixing up the reagents of other test systems or lots (see also 3.).

HEALTH AND SAFETY INFORMATION

* The local occupational safety and health regulations have to be regarded.

* Reagents of human origin have been tested and found to be negative for HBsAg, for antibodies to HIV-1/2 and to HCV. Nevertheless, it is strongly recommended that these materials as well as those of animal origin (see kit contents), should be handled as potentially infectious and used with all necessary precautions.

DISPOSAL CONSIDERATIONS

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.

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