VIROTECH Bordetella pertussis Toxin (PT) IgM ELISA
(B. pertussis PT IgM ELISA)

Order No.: EC215M00

Color Coding: blue metallic/transparent

FOR IN VITRO DIAGNOSIS ONLY

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Contents

1. Intended Use

The Pertussis Toxin ELISA is intended for the semiquantitative and qualitative detection of IgM-antibodies in human serum. It is intended for the detection of an acute or recent infection.

2. Diagnostic Relevance

The main agent of the genus *Bordetella*, *B. pertussis*, causes the clinical picture of whooping-cough. Milder forms are caused by *B. parapertussis,* those are not detected with the Pertussis Toxin ELISA.

The Pertussis Toxin is of significant importance for the pathogenesis of whooping cough. It is a real exotoxin responsible for many physiological, immunological and pharmacological effects. In contrast to other exotoxins of the species *Bordetella*, that show high cross-reactivities in serum diagnostics, the Pertussis Toxin is high-specific (4).

During primary infection, the IgM-antibodies can be detected at the earliest 5-10 days after the beginning of the convulsive stage and persist for 6-12 weeks; they are the expression of an acute disease. IgA-antibodies can be detected 11 days after disease started at the earliest. IgA antibodies can persist 6-24 months. They are also developed in vaccinated adults during a natural re-infection (without clinical disease) and are therefore found in healthy adults as well. Infected infants up to an age of 12 months do usually not develop IgA antibodies against Pertussis Toxin. Infants between 1-4 years rarely develop IgA antibodies against Pertussis Toxin, at an age between 5-10 years they develop only very small concentrations of IgA antibodies against Pertussis Toxin (6). In this case the detection of specific IgM can be a notice for a recent infection (3). IgG antibodies occur 2-3 weeks after beginning disease in the serum at the earliest. Re-infections are marked by increased antitoxin-IgG- and -IgA-antibodies as a rule. IgG- and secrete-IgA-antibodies are, beside the specific sensibilised T-lymphocytes, the carrier of the long-term-immunity (2).

The pertussis serology cannot replace antigen detection, but should ber performed in addition. The anti-pertussis antibodies are produced later in comparison to other infectious diseases.

3. Test Principle

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

4. Package Contents (IgM Testkit)

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7,2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
4. **IgM negative Control, 2000µl,** human serum with protein-stabilizer and preservative, ready to use
5. **IgM cut-off Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgM positive Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
7. **IgM-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
8. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
9. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

5. Storage and Shelflife of the Testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.

3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

Material	Status	Storage	Shelflife
Test Samples	Diluted	+2 to +8°C	max. 6h
	Undiluted	+2 to +8°C	1 week
Controls	After Opening	+2 to +8°C	3 months
Microtitreplate	After Opening	+2 to +8° (storage in the provided bag with desiccant bag)	3 months
Rheumatoid factor - Absorbent	Undiluted, After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	1 week
Conjugate	After Opening	+2 to +8°C (protect from light)	3 months
Tetramethylbenzidine	After Opening	+2 to +8°C (protect from light)	3 months
Stop Solution	After Opening	+2 to +8°C	3 months
Washing Solution	After Opening	+2 to +8°C	3 months
	Final Dilution (ready-to-use)	+2 to +25°C	4 weeks

6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. Test Procedure

Working exactly referring to the VIROTECH Diagnostics user manual is the prerequisite for obtaining correct results.

8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.

Always prepare patient-dilution freshly.

For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

8.2 Preparation of Reagents

The VIROTECH Diagnostics System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The

ready to use controls (positive control, negative control, cut-off control) are **parameter specific** and **only to use** with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.
5. High IgG-titer or rheumatoid factors may disturb the specific detection of IgM-antibodies and may lead to false positive resp. false negative results. **For a correct IgM-determination it is therefore necessary to pre-treat the sera with RF-SorboTech** (VIROTECH adsorbent). For IgM-controls a pre-absorbent treatment is not necessary.

8.3 VIROTECH ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgM-positive, negative and cut-off control as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off control a double insertion is absolutely necessary. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 – 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

8.4 Usage of ELISA processors

All VIROTECH Diagnostics ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

VIROTECH Diagnostics recommends the following procedure:

1. VIROTECH Diagnostics recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.

9. Test Evaluation

The ready to use controls serve for a semiquantitative determination of specific IgM-antibodies. Their concentration can be expressed in VIROTECH units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control

a) OD-values

The OD of the blank should be < 0.15.

The OD-value of the negative control should be lower than the OD-value mentioned in the Quality Control Certificate. The OD-values of the positive control as well as of the cut-off control should be above the OD-values mentioned in the Quality Control Certificate.

b) VIROTECH Units (VE)

The VIROTECH Units (VE) of the cut-off control are defined as 10 VE. The calculated VE of the positive control should be within the range mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the VIROTECH Units (VE)

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

$$VE\ (\text{positive control}) = \frac{OD\ (\text{positive control})}{OD\ (\text{cut - off control})} \times 10$$

$$VE\ (\text{patient serum}) = \frac{OD\ (\text{patient serum})}{OD\ (\text{cut - off control})} \times 10$$

9.3 Interpretation Scheme IgM

Result (VE)	Evaluation
< 9,0	negative
9,0 - 11,0	borderline
> 11,0	positive

1. IgM-antibodies are not always developed and are therefore a less reliable marker for a *Bordetella pertussis* infection than IgG-antibodies.
2. If the measured values are above the defined borderline range, they are considered to be positive.
3. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 – 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.
4. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.
5. At a borderline IgM result and the presence of an IgG result <18 VE, a second serum sample is necessary to check for an acute infection.

9.4 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.

10. Performance Data

10.1 Prevalence (Expectet Values)

The following table shows the results of the examination of 80 blood bank sera:

	IgM
negative	79
borderline	1
positive	0

10.2 Intra-assay-Coefficient of Variation (Repeatability)

In one assay, strips of different plates of one batch have been tested with the same serum sample. The obtained coefficient of variation for IgM is < 9%.

10.3 Inter-assay-Coefficient of Variation (Reproducibility)

In 10 independent testruns 3 sera have been tested in different laboratories of different persons.
The obtained variation coefficient values are lower than 15%.

11. Literature

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Preparation of Patient Samples and Washing Solution

▼ **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

▼ **IgM-Samples – Dilution**
1:101
Rheumafactor-absorption with RF-SorboTech
e.g.:
5 µl serum/plasma + 450 µl Dilution Buffer +
1 drop RF-SorboTech, incubate for 15 min. at room temperature.

Testprocedure

Samples Incubation	**30 minutes at 37°C**	**100 µl Patient Samples** blank value (Dilution Buffer) and controls
⇩		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Conjugate Incubation	**30 minutes at 37°C**	**100 µl Conjugate** IgM
⇩		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Substrate Incubation	**30 minutes at 37°C**	**100 µl Substrate**
⇩		
Stopping		**50 µl Stopping Solution** shake carefully
⇩		
Measure Extinctions		**Photometer at 450/620nm** **(Reference Wavelength 620-690nm)**