

**VIROTECH Bordetella pertussis Toxin (PT) IgG ELISA
(B. pertussis PT IgG ELISA)**

Order No.: EC215G00

B. pertussis PT IgG Quant.-Set

Order No.: EN215Q60

**VIROTECH Bordetella pertussis Toxin (PT) IgA ELISA
(B. pertussis PT IgA ELISA)**

Order No.: EC215A00

Color Coding:

IgG: silver/dark blue

IgA: silver/black

FOR IN VITRO DIAGNOSIS ONLY

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1. Intended Use

The Pertussis Toxin ELISA is intended for the semiquantitative and qualitative detection of IgG or IgA-antibodies in human serum.

It is intended for the detection of an acute or recent infection respectively for the detection of vaccination antibodies (check of vaccination success). It is also possible to quantify the IgG in international units per ml (IU/ml), by using the IgG Quantification Set (EN215Q60), which is supplied separately.

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 be performed in addition. The anti-pertussis antibodies are produced later in comparison to other infectious diseases.

With the WHO (World Health Organization) International Standard Pertussis Antiserum (NIBSC (National Institute for Biological Standards and Control) code: 06/140), a reference serum enabling the standardised and precise quantification of the anti-PT IgG concentration of a patient serum in International Units/ml has been in existence since 2009. The *Bordetella* reference centres in several countries have suggested that this standard serum should be used for a two cut-off system, with a lower limit of 40/ 50 IU/ml and an upper limit of 100/ 120 IU/ml(11, 12, 13).

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

4.1 IgG 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. **IgG negative Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
5. **IgG cut-off Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgG positive Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
7. **IgG-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer 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

4.2 IgG Quantification Set

1. **IgG calibration control, 2000 µl**, human serum with protein stabilisers and preservative, ready-to-use
2. **IgG weakly reactive control, 2000 µl**, human serum with protein stabilisers and preservative, ready-to-use
3. **IgG strongly reactive control, 2000 µl**, human serum with protein stabilisers and preservative, ready-to-use

4.3 IgA 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. **IgA negative Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
5. **IgA cut-off Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgA positive Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
7. **IgA-Conjugate 2 (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 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. **In order to determine the pertussis toxin IgA correctly, it is necessary to pre-treat the sera using RF-SorboTech (VIROTECH adsorption medium).** Pre-adsorption is omitted in IgA controls.

8.3 VIROTECH ELISA Test Procedure

1. For each test batch, pipette 100µl each of the ready-to-use dilution buffer (blank), the controls and the diluted patient sera. We recommend the use of duplicates (blank, controls and patient sera). It is absolutely essential to use duplicates for the cut-off and calibration control. Working dilution of the 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!

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. Qualitative and semiquantitative test evaluation

The ready to use controls serve for a semiquantitative determination of specific IgG- and IgA-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-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) VIROTECH Units (VE)

The VIROTECH Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges 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.

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

9.3 Interpretation Scheme IgG

The VIROTECH units (VE) of the Pertussis Toxin IgG ELISA (enzyme-linked immunosorbent assay) have been calibrated using the WHO International Standard. This leads to the following calibration between VIROTECH Units (VE) and International Units per ml (IU/ml) for IgG (7).

IU/ml (WHO)	VE	IgG- antibodies	Interpretation
	< 9	negative	<p>⇒ <i>no increased antibody-titer against Pertussis-Toxin:</i></p> <ul style="list-style-type: none"> No suspicion of a <i>B. pertussis</i>-infection <p>In case of clinical symptoms a follow up control or a differential diagnosis is recommended.</p>
36-44	9 – 11	borderline	<p>⇒ <i>increased antibody-titer against Pertussis-Toxin:</i></p> <ul style="list-style-type: none"> Persistent antibodies of a recent infection antibodies of a starting immune response vaccination antibodies

IU/ml (WHO)	VE	IgG- antibodies	Interpretation
	> 11	positive	⇒ <i>significant high antibody-titer against Pertussis-Toxin</i> <ul style="list-style-type: none"> Note towards an acute or recent infection Detection of vaccine antibodies: Absolutely consider vaccine-management, as the test is not able to differentiate between vaccine antibodies and antibodies of an infection.
≥ 100	≥ 17	infection	⇒ <i>Significant high antibody-titer against Pertussis-Toxin, an argument for an acute infection if the last vaccination is longer than 12 months ago.</i>

9.4 Interpretation Scheme IgA

The Pertussis Toxin IgA ELISA has been adjusted to the WHO International Standard. This gives the correlation in the evaluation between VIROTECH Units (VE) and International Units per Milliliter (IU/ml) for IgA (11, 12).

IU/ml (WHO)	VE	IgA antibodies	Interpretation
< 12 IU/ml	< 9	negative	⇒ <i>no increased Ab titre to pertussis toxin:</i> <ul style="list-style-type: none"> No suspicion of <i>B. pertussis</i> infection If there are clinical symptoms, repeat measurements later or clarify via differential diagnosis.
	9 – 11	borderline	⇒ <i>increased Ab titre to pertussis toxin:</i> <ul style="list-style-type: none"> Persisting Ab from a previous infection Ab from the initial stages of an immune response Vaccine antibodies
≥ 12 IU/ml	> 11	positive	⇒ <i>significantly increased Ab titre to pertussis toxin:</i> Accompanied by positive IgG Ab titre (> 11 VE): <ul style="list-style-type: none"> Evidence for new or recent infection Detection of vaccine antibodies: Essential to consider vaccine management, as the test cannot distinguish between vaccine antibodies and infection antibodies. Accompanied by negative or threshold IgG Ab titres (≤ 11 VE): <ul style="list-style-type: none"> Repeat measurements later.

Notice: IgA-antibodies are not always developed and are therefore a less reliable marker for a *Bordetella pertussis* infection than IgG-antibodies.

- Absolutely consider vaccine-management, as the test is not able to differentiate between vaccine antibodies and antibodies of an infection.
- If the measured values are above the defined borderline range, they are considered to be positive.
- 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.
- 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.
- At a borderline IgA result and the presence of an IgG result <17 VE, a second serum sample is necessary to check for an acute infection.

9.5 Limits of the Test

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

10. Quantitative IgG test evaluation in IU/ml

The ready-to-use calibration control is separately available with the IgG Quantification Set (EN215Q60) and is used for the quantitative determination in IU/ml of anti-PT IgG antibodies in patient serum. The calibration control corrects for fluctuations from the test performance. The mean of the OD values is used for the calculation.

10.1 Control of test function

a) OD values

The OD value of the blank should be <0.15.

The OD value of the calibration control must lie within the range given in the corresponding certificate.

b) IU/ml

The anti-PT IgG concentrations (IU/ml) of the weakly reactive control and of the strongly reactive control must lie within the ranges given in the quality control certificate.

If the requirements are not fulfilled (OD values, IU/ml), the test must be repeated.

10.2 Calculation of the quantitative results in International Units per ml (IU/ml)

The extinction of the blank (450/620nm) must be subtracted from all extinctions.

Using the VIROTECH IgG Quantification Set, the patient sera are quantified by correlation with the WHO International Standard. Extensive tests have been performed with each plate batch. This has led to a standard non-linear regression curve, expressed mathematically by the following formula (14):

$$\text{IU/ml} = \exp(-(\ln((D-A)/((OD \text{ corr})-A)-1)-B)/C)$$

Where

- A: expected OD with an anti-PT IgG concentration of 0
- B: gradient
- C: point of inflexion
- D: expected OD at an infinitely high anti-PT IgG concentration
- OD corr: corrected OD of the patient serum

To correct for fluctuations within test processing, the measured OD of the patient serum is corrected with a calibration control.

$$\text{OD corr} = \text{OD Patient serum} * \frac{\text{OD Calibration control given}}{\text{OD Calibration control measured}}$$

The values of parameters A, B, C and D, as well as the given value for the OD of the calibration control, are to be found in the certificate.

Determination of the IU/ml value

The IU/ml can be determined with a program that can be ordered from VIROTECH. Alternatively, an evaluation template for common table calculations can be supplied.

The borderline range for the quantification with the VIROTECH Pertussis Toxin IgG Quantification Set is defined as being from ≥ 40 IU/ml to < 100 IU/ml, corresponding to the VE range from ≥ 10 VE to < 17 VE.

The quantifiable range lies from 5 IU/ml to 500 IU/ml.

10.3 Interpretation scheme for IgG

The international units (IU/ml) of the Pertussis Toxin IgG ELISAs were calibrated with WHO International Standard 335. The interpretation is in accordance with the recommendations of European Reference Centres (7, 11, 12, 13, 15).

IU/ml (WHO)	Interpretation
< 40	No evidence for recent contact with the pathogen
≥ 40 to < 100	Doubtful result. Repeat measurements later or determine IgA-anti-PT: <ul style="list-style-type: none"> - IgA-anti-PT ≤ 11 VE (corresponding to < 12 IU/ml): No evidence for recent pathogen contact - IgA-anti-PT > 11 VE (corresponding to ≥ 12 IU/ml): Evidence for recent pathogen contact, assuming that the last vaccination was more than 12 months previously - Pay attention to vaccine management!
≥ 100	Evidence for recent pathogen contact, on the assumption that the last vaccination was more than 12 months previously. Check vaccination management!

11. Performance Data

11.1 Sensitivity and Specificity

In an internal study, 151 sera with suspected *Bordetella pertussis* infection were tested for anti-PT IgG. These sera have been predefined in Neutralisation test (NT) by a former reference centre. The VIROTECH Bordetella pertussis LINE (Line Immuno Assay), has been used as comparative test.

The results are summarised in the following table.

Sera Collective (n= 151)

VIROTECH LINE IgG	VIROTECH Pertussis Toxin ELISA IgG		
	negative	borderline	positive
negative	76	1	1
borderline	6	1	7
positive	2	1	56

Borderline results have not been considered for the calculation of the sensitivity and specificity. Relative to Bordetella pertussis LINE, the sensitivity for IgG was 96.6 % and the specificity 98.7 %.

11.2 Cross Reactivity

In order to test for any cross-reaction between VIROTECH Pertussis Toxin ELISA and antibodies from respiratory diseases, 37 sera was tested for IgG and 33 sera was tested for IgA.

The results are shown in the following table:

	IgG	IgA
negative	29	33
borderline	4	0
positive	4	0

The result shows that the VIROTECH Pertussis Toxin ELISA is a very good tool for the differential diagnostic usage.

11.3 Prevalence (Expectet Values)

The following table shows the test results of blood donators for 80 IgG samples and 78 IgA samples.

	IgG	IgA
negative	73	78
borderline	3	0
positive	4	0

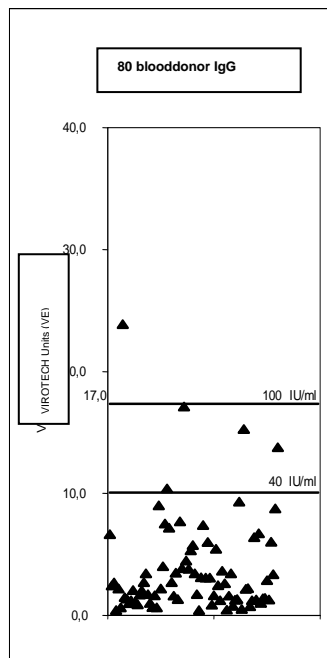
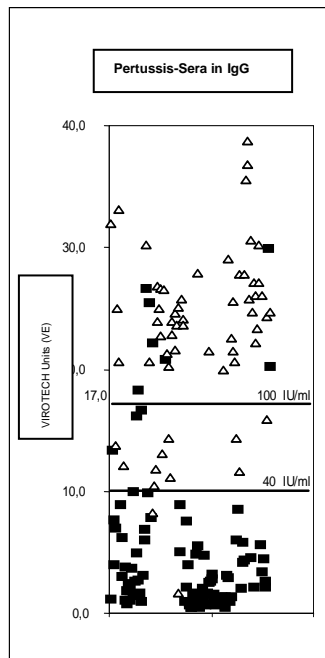
11.4 Intra-assay-Coefficient of Variation (Repeatability)

In this assay, strips from various plates of a batch were tested in a chessboard pattern with two sera. The coefficients of variation are as followed: < 9% for IgG and < 15% for IgA.

11.5 Inter-assay-Coefficient of Variation (Reproducibility)

A minimum of 3 sera were tested in minimum 10 independent test batches on 3 different test days. This gave a coefficient of variation of $\leq 15\%$.

11.6 Distribution of the antibody concentrations (in VE) of sera with/without suspected Pertussis



The main part of the negative Pertussis-Sera in IgG is found below the 10VE-limit, the main part of the positive ones above the 17VE-limit.

In the area between 10 and 17 VE, positive sera are found, whose antibodies may result from post or just starting infection.

Significant high antibody-titer responsible for an acute infection, if the vaccination is longer than 12 months ago, are found above 17 VE.

The main part of the negative sera of blooddonors is found below the 10 VE-limit.

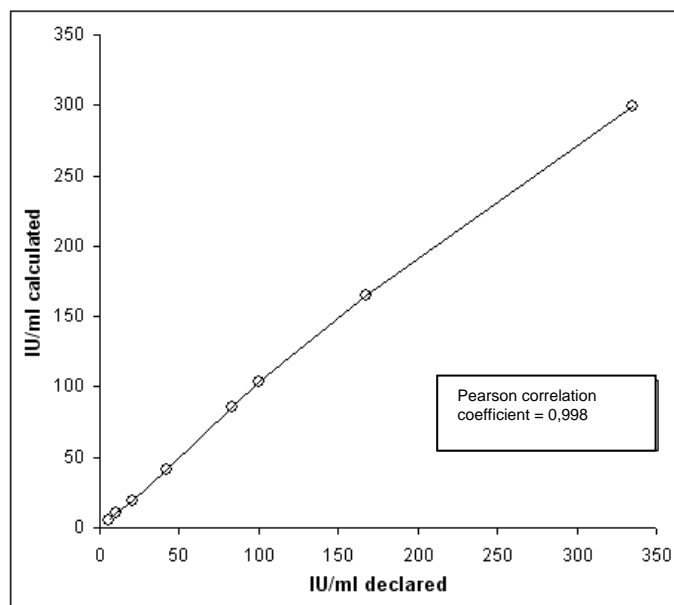
△ in LINE¹ predefined positive sera
■ in LINE¹ predefined negative sera

▲ blooddonors

¹ Line-Immuno-Assay of VIROTECH

11.7 Correlation between declared and measured IU/ml

The following diagram shows the correlation of the declared IU/ml of several dilutions of the WHO International Standard with the IU/ml determined using the VIROTECH Pertussis Toxin IgG ELISA. The calculated Pearson correlation coefficient demonstrates the very good agreement between the calculated and declared values.



12. Literature

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Preparation of Patient Samples and Washing Solution

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

**IgG-Samples – Dilution
1:101**

e.g.:
10 µl serum/plasma + 1000 µl Dilution Buffer
(Serum Dilution Buffer is ready to use)

**IgA-Samples – Dilution
1:100**

e.g.:
5 µl serum/plasma + 450 µl Dilution Buffer +
1 drop RF-SorboTech, incubate for 15 min. at room
temperature.

Testprocedure

