VIROTECH Bordetella pertussis + CatACT IgG LINE Immunoblot

(B. pertussis + CatACT IgG LINE-32)

Order No.: WE116G32

(B. pertussis + CatACT IgG LINE-96)

Order No.: WE116G96

VIROTECH Bordetella pertussis + CatACT IgA LINE Immunoblot

(B. pertussis + CatACT IgA LINE-32)

Order No.: WE116A32

(B. pertussis + CatACT IgA LINE-96)

Order No.: WE116A96

FOR IN VITRO DIAGNOSIS ONLY

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REV 10 / VIROTECH B. pertussis + CatACT IgA & IgG LINE Immunoblot GB

Contents

1. Intended Use

Line Immunoblot Testkit for the qualitative detection of *Bordetella pertussis* specific IgG- respectively IgA- antibodies in human serum. The kit is used to detect a new, recent or long past infection with Bordetella pertussis or for differential diagnosis of longer lasting clinical manifestations with uncharacterised coughing. By virtue of the simultaneous detection of specific antibodies against pertussis-toxin (PT) and the catalytic part of the adenylate cyclase toxins (CatACT), the distinction between a Bordetella pertussis infection and an inoculation can be facilitated in most cases.

Furthermore, the Line Immunoblot may provide evidence of possible infection with B. parapertussis. The absence of specific antibodies against PT and simultaneous presence of antibodies against CatACT [genus-specific] (17) and FHA may be regarded as evidence of B. parapertussis infection.

2. Diagnostic Meaning

B. pertussis , the main protagonist of the species *Bordetella* , brings about the clinical symptoms of whooping cough. Milder forms are caused by *B. parapertussis*.

At a primary infection IgM-antibodes can be found 5-10 days after beginning of the stage convulsivum at the earliest and persist for 6-12 weeks; they are the expression of an acute disease. IgA antibodies can be detected as early as 11 days after onset of the disease and may persist for 6-24 months. They are developed also by vaccinated people during a natural re-infection (without clinical disease) and can therefore also be found in healthy adults. Infected infants up to the age of 12 months do not, as a rule, develop IgA-antibodies against Pertussis-Toxin. Infants between 1 and 4 years develop IgA antibodies against Pertussis-Toxin rarely whilst infants between 5 and 10 years develop IgA-antibodies against Pertussis-Toxin only in very low concentrations (1). IgA- and IgM-antibodies are not always developed and thus are a less reliable marker for a *Bordetella pertussis*-infection than IgG-antibodies. The production of antibodies begins at different times in different patients, but as early as in the second week of the convulsivum stage (IgG is present as early as 2-3 weeks after start of the infection) and maximum production is achieved in six to eight weeks after the start of paroxysmal coughing. For this reason, identification of an initial serum is recommended for Bordetella diagnosis that is taken 2 weeks after the start of paroxysmal coughing and a second serum 3 to 5 weeks thereafter.

Reinfections are normally identified by increased antitoxin IgG and IgA antibodies. (2).

Pertussis-Toxin, a true exotoxin responsible for many physiological and immunological effects, is of crucial importance for pathogenesis of pertussis. In contrast to other exotoxins of the *Bordetella* genus that exhibit high cross-reactivity, the Pertussis-Toxin is highly specific (3, 14, 15).

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. Principle of Test

Proteins from *B. pertussis* are transferred to a nitrocellulose membrane by a special spray method. The nitrocellulosis membrane is then cut into single strips.

Incubation of the antigen-coated nitrocellulose strips with samples of human serum or plasma permits the detection of specific antibodies. These antibodies develop immuncomplexes with the antigen fixed on the test strip. After removing the unbound antibodies by washing steps, the single nitrocellulose-strips are incubated with alcalic phosphatasis conjugated anti-human IgG- respectively IgA-antibodies. After unbound conjugated antibodies have been removed by a further washing step, a visualisation of the anitgen/antibody-complex (of the bound antibodies) is accomplished by the addition of a non-coloured substrate, which forms blue-violette precipitates at each site (Ăantigen bandsñ) where the conjugated anti-human antibodies have bound. The enzyme/substrate-reaction is stopped through washing the nitrocellulose-strips with aqua dest./deionised. Depending on the observed band pattern one can interprete the presence of specific IgG- respectively IgA-antibodies.

4. Package Contents

4.1 Kit for 32 determinations

1.	**IgG resp. IgA *Nitrocellulose test* strips** with sprayed antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use**.**	**1x**	32 strips
2.	**IgG resp. IgA Cut off Controll**, human serum, prediluted For use of the Cut-off Control refer to point 11. And point 12.	**1x**	1.0 ml
3.	**Dilution-/ washbuffer, pH 7.3 (10x conc.), with Tris and preservative**	**2x**	**50 ml**
4.	**IgG- resp. IgA- Conjugate** (100x conc.) **Anti-human-**(goat)-Alcalic Phosphatasis, with preservative	**1x**	0.7 ml
5.	**Substrate** (BCIP/NBT), ready to use	**1x**	57 ml
6.	**Evaluation Record sheet** for the notation and deposit of the results	**1x**	1 pcs.

4.2 Kit for 96 determinations

1.	**IgG resp. IgA *Nitrocellulose test* strips** with sprayed antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use**.**	**3x**	32 strips
2.	**IgG resp. IgA Cut off Controll**, human serum, prediluted For use of the Cut-off Control refer to point 11. And point 12.	**2x**	1.0 ml
3.	**Dilution-/ washbuffer, pH 7.3 (10x conc.), with Tris and preservative**	**4x**	**50 ml**
4.	**IgG- resp. IgA- Conjugate** (100x conc.) **Anti-human-**(goat)-Alcalic Phosphatasis, with preservative	**3x**	0.7 ml
5.	**Substrate** (BCIP/NBT), ready to use	**3x**	57 ml
6.	**Evaluation Record sheet** for the notation and deposit of the results	**3x**	1 pcs.

Also available on request:

IgG or IgA- Positive control, human serum, prediluted, 0.5 ml.
The positive bands > For the cut--off band, refer to the certificate supplied with the kit.
(Order No.: IgG: WE116P60 or IgA: WE116P40)

IgG/IgA- Negative control, human serum, prediluted, 0.5 ml.
The negative control shows no bands or no bands relevant to the evaluation. > Cut-off band.
(Order No.: IgG/IgA: WE116N20)

5. Storage and Stability

Store test kit at 2-8°C. The shelf life of the single components is mentioned on the relevant label; for shelf life of the Kit please refer to the Quality Control Certificate.

1. Do not expose the single kit components to high temperature nor freeze them.
2. Do not use the kit reagents after their expiring date.
3. Do not expose reagents to strong light during storage or incubation.
4. The BCIP/NBT-substrate solution is sensitive to light and has to be stored in dark.

5. **Nitrocellulose test strips :** Use strips immediately after taken out of the bag. Close bag with the not required strips again savely and store at 2-8°C. When putting the results into archives please take care that the nitrocellulose test strips and templates are protected against direct sunlight, to avoid fading of the bands.

Material	Status	Storage	Shelflife
Test Samples	Undiluted	+2 to +8°C	1 week
Test Strips	After Opening	+2 to +8°C (stored in supplied bag)	3 months
Controls	After Opening	+2 to +8°C	3 months
Conjugate	After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	ca. 6h
Substrate	After Opening	+2 to +8°C (protect from light)	3 months
Washing Solution	After Opening	+2 to +8°C (protect from light)	3 months
	Final Dilution (ready-to-use)	+2 to +8°C	4 weeks
	Final Dilution (ready-to-use)	*or* room temperature	2 weeks

6. Precautions and Warnings

1. Only sera, that have been tested and found to be negative for HIV1-ab, HIV2-ab, HCV-ab and Hepatitis-B-surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls and conjugate as well as the antigen strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Use plastic foreceps and wear protective gloves when handling the Immunoblot.
3. Please follow the local valid waste disposal regulations.
4. The incubation baths are designed by the manufacturer for a single use. The reuse of the incubation baths is at the risk of the user. If they are to be reused we recommend that after use the incubation baths be disinfected for several hours in 1% sodium hypochlorite solution and then rinsed thoroughly with tap water followed by distilled or deionized water.

7. Additionally required material (not supplied)

1. Incubation tray (if required available with order no.: WE300.08)
2. Rocking platform (vertical not centrifugal)
3. A wash bottle for stopping
4. Pipette or handwasher
5. Micro-pipettes 5 µl - 1500 µl
6. Pipette filler
7. Test tubes, 2-20 ml volume
8. Plastic foreceps
9. Aqua dest. or deionised water
10. Filter paper

8. Examination Material

Either serum and plasma may be used as test materials, even when the package leaflet only mentions serum. Plasma samples may contain any anticoagulant.

9. Test Procedure

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

9.1 Preparation of Samples

1. 15 µl serum or plasma are needed for each patient sample.
2. Blood samples should be taken separately by venous puncture. Serum is separated after complete coagulation (not applicable to plasma). If they are to be stored longer sera have to be frozen at -20°C.
3. Repeated freezing and thawing should be avoided.
4. Sera that are heat-inactivated, lipaemic, haemolytic or microbiologically contaminated, may lead to faulty results and shall therefore not be used.
5. Do not use turbid samples (especially after thawing), centrifuge if necessary (5 minutes at 1000sg), pipette clear supernatant and use in testing.

9.2 Preparation of Reagents

1. To facilitate routine laboratory work, all LINEs can be processed in a single test run with the same incubation times and the same component - when these are independent of the parameters and batches. The cut-off controls now have parameter and batch specific values.
2. Bring the corresponding concentrate to room temperature (20-25°C) before preparing the dilution. Use only high quality Aqua dest./deionised and bring up to room temperature (20-25°C) before usage.
3. Mix dilutions well before starting the test.
4. **Dilution-/Washbuffer:**
   The dilution-/washbuffer is provided as a 10-fold concentrate. Dilute the dilution-/washbuffer concentrate 1:10 with distilled or deionised water (10ml/50ml/100ml concentrate + 90ml/450ml/900ml distilled or deionised water), mix well. Both the concentrated and the diluted dilution/washing buffer may exhibit a yellow colouration. This colouration does not influence the stability of the dilution/washing buffer or the function or the reliability of the diagnostic test.
5. **IgG resp. IgA conjugate**
   Dilute the conjugate 1 + 100 with finally diluted dilution/washing buffer and mix thoroughly. 1.5 ml conjugate working solution is required for each serum sample. See conjugated dilution table (item: "Test Procedure")**.**
6. **Substrate Solution**
   The substrate solution is delivered ready-to-use.

9.3 Immunoblot Test Procedure

Attention: **For the correct performance and evaluation of the B. pertussis + CatACT LINEs, each test run should include the appropriate parameter and batch-specific cut-off controls.**

For a secure *Bordetella pertussis* diagnostic the LINE shall be proceeded in IgG and IgA.

1. Test has to be proceeded at room temperature.
2. For each sample put 1 strip into the channel of a clean incubation tray. Hold strip only at the marked upper end.
3. Pipette 1,5ml ready to use **dilution-/ washbuffer** each and put onto the rocking platform. Take care that the antigen strips are consistently covered with liquid, the strips must not dry out during the whole test procedure.
4. The solid antigen strips are being moistured completely within one minute and can be incubated in supine, lateral position or face-down position.

5. 15 µl patient serum or plasma **or** 100 µl of the cut-off or positive / negative control **added by pipetting, if at all possible at the upper marked end of the strip.** Incubate patient serum and control for **30 minutes** on the rocking platform. Take care that during pipetting and following pour away no cross-contamination of the single patient samples occur.
6. Aspirate or carefully pour away the liquid out of the channels completely. During the pour away of the liquid, the antigen strips remain at the bottom of the channel. Drain the remaining liquid onto a cellulosis paper.
7. **Washing** of strips: Incubate with 1,5 ml ready to use dilution-/washbuffer each for **3 x 5 minutes** on the rocking platform. Pour away or aspirate washing buffer always completely. Before ending of the last washing step, prepare the needed amount of fresh conjugate dilution (refer to table).
8. Aspirate or pour away the liquid completely out of the channels (please refer to point 6).
9. Pipette 1,5 ml of the prepared **conjugate dilution** each into the corresponding incubation channel and incubate for **30 minutes** on the rocking platform.
10. Pour away or aspirate liquid completely out of the channels.
11. **Washing** of the strips: Incubate with 1,5 ml ready to use dilution-/washbuffer each for **3 x 5 minutes** on the rocking platform. Pour away or aspirate the washbuffer always completely. Afterwards rinse **1 x 1 minute** with **Aqua dest./deionised**.
12. Pour away or aspirate the liquid completely out of the channels (refer to point 6).
13. Pipette 1,5 ml ready to use **substrate solution** each into the channels and allow to develop **10** ± **3 minutes** on the rocking platform.
14. **Stop** the color reaction by pouring away the substrate solution. Afterwards wash the strips without incubation in between for **3 x** with 1,5 ml **Aqua dest./deionised** each.
15. Pour away the aqua dest./deionised and let the strip dry on a clean cellulosis paper. The background-coloring, that may be observed on the moistured antigen strips disappears completely when the strips are completely dry. Solid antigen strips need a little longer than the conventional antigen strips until they are completely dry.
16. Use the included calculation protocol for the interpretation. The inscription of the high-specific band on the protocol sheet makes the interpretation of the patient samples easier for you.

For test procedure scheme pls. refer to last page

9.4 Use of Immunoblot-processors

The following instruments have been validated for the automatic processing of the Blots and LINEs: Apollo and Profiblot. All commercially available Blot machines are suitable in principle.

10. Interpretation of Results

For a secure interpretation each LINE is fitted out with two controls:

1. **Serum control:**
   Only after the incubation with patient serum the serum incubation band appears below the markline.
2. **Conjugate control:**
   The *Bordetella pertussis* LINE strip is fitted out with an IgG- resp. IgM conjugate control band.

The test procedure is valid, if the serum control as well as the internal conjugate control appears clearly visible on the developed antigen strip.
Please refer to the protocol sheet for the information of the exact position of the serum- and the conjugate control.

10.1 Interpretation of the patient samples

Please refer to the protocol sheet for position and denotation of reactive bands.

IgG and IgA bands: FHA, CatACT, PT

10.2 Usage of the cut-off control

The intensity of the PT bands of the cut-off control is used for semiquantitative analysis of all occurring bands:

Occurring Bands	Analysis of Band Intensities
> Cut-off band (PT)	positive (+)
= Cut-off band (PT)	borderline (+/-)
< Cut-off band (PT)	negative (-)

10.3 Meaning of the antigens

Listing of the purified (native PT and FHA) and recombinant (CatACT) Bordetella pertussis antigens used and originating from the Tohama Phase I strain.

Antigen / Designation	Meaning of the Antigens	Specificity of the Antibody in the LINE
PT 28kDa	**Pertussis toxin (PT)** is an exotoxin derived only from B. pertussis and is therefore highly specific for this pathogen. It consists of an enzymatically active A-subunit (subunit S1) and a receptor-binding B-subunit. Components of the acellular vaccine and antibodies against PT in vaccination sera exhibit the greatest correlation with protection against B. pertussis infection. For diagnosis of Bordetella in IgG via an individual serum, PT represents the antigen with the highest sensitivity and specificity, each exceeding 98%. Pertussis toxin may therefore be considered a marker protein of B. pertussis infection. In IgA and IgM, the antibody response against PT is present only in approximately 40-50% of the pertussis cases (4, 5).	Highly specific for *B. pertussis*
CatACT 43kDa	**Adenylate cyclase toxin (ACT)** is an antigen not found in B. pertussis vaccines. It is an essential virulence factor of B. pertussis (6). The cross-reactivity of the total ACT protein with other members of the RTX toxin family - including the haemolysin of E. coli (7, 8, 9, 10) - albeit lacking in the enzymatic unit of adenylate cyclase, is generally known. For this reason the B. pertussis + CatACT LINE uses only the N-terminal, 400 AA long residue of the ACT antigen (referred to as CatACT), which contains catalytic domains specific for Bordetella sp. For IgG, the CatACT is the best infection marker for serological diagnosis, which is not affected by the vaccination status. At the time of diagnosis, 68.0% of the culture-positive patients were seropositive for CatACT in the IgG. It has been shown that CatACT is the second most sensitive marker after PT. (11) Therefore, the simultaneous detection of antibodies against CatACT and PT suggest the presence of an acute or recently passed infection.	Specific for *Bordetella sp.*
FHA 220kDa	**Filamentous Hemagglutinin** (FHA) is a surface-associated protein of *Bordetella pertussis*. The pathogen uses it as an important adherence protein (4). The antibody response to FHA is very high particularly in the IgG with approx. 80-90%; in the IgA and IgM, it lies at approx. 50-60% (4, 5). In the meantime, several independent studies (3, 12, 13) have been able to show the presence of cross-reactivity of FHA	Less specific

	from *Bordetella pertussis, Bordetella parapertussis* and among other bacterial pathogens.	

10.4 Interpretation criteria

The interpretation of the serological result shall always include the clinical picture, epidemiological data and further diagnostical parameter.

Recommended IgG and IgA analysis

PT	CatACT	Meaning	Interpretation B. pertussis
-	**-** **+/-** **+**	No indication of a *Bordetella pertussis* infection. Possible evidence of B. parapertussis infection (see below)	**negative**
+/-	**-** **+/-** **+**	The possibility of a very early or long past *B. pertussis* infection can be determined. Check by means of a second serum is recommended.	**borderline**
+	**-**	Indication of a new or recent B*ordetella pertussis* infection. **Review** vaccination status	**positive**
+	**+/-** **+**	Clear indication of a new or recent B*ordetella pertussis* infection.	

Additional evidence of B. parapertussis infection in IgG and IgA:

PT	CatACT	FHA	Significance	Interpretation for B. pertussis
-	+/- +	+	possible evidence of *B. parapertussis* infection	negative

A CatACT and FHA positive result can be interpreted as an indicator for a B. parapertussis infection in case of a lacking PT response.

To confirm the suspicion of a *B. parapertussis* infection, we recommend testing a second serum 7 days later; at that time antibodies against PT should still not be detectable. Alternatively, a PCR for *B. parapertussis* may be carried out.

FHA band: The mere presence of antibodies against the unspecific group antigen FHA does not permit a distinction between an infection due to *Bordetella pertussis* or *Bordetella parapertussis*. This could also result from a cross reaction of the FHA with *Haemophilus influenzae* or other pathogens or from persistent antibodies (vaccine antibodies or antibodies of a previous infection).
In case of questions about serological Bordetella results that take FHA into consideration, the FHA band of the LINE may also be evaluated; see *B. parapertussis*. For this purpose the FHA band will be appraised via the intensity of the PT band of the cut-off control:

- Cut off: positive / = Cut off: limit value / < Cut off: negative

Note: IgA and IgM antibodies are not always formed and are therefore a less reliable marker for a *Bordetella pertussis* infection than IgG antibodies.

10.5 Limits of the Test

1. A negative LINE result does not completely exclude the possibility of an infection with *Bordetella pertussis*. The sample may be taken before the occurance of antibodies, or the antibody titre exists below the detection limit of the test.
2. Due to a long-term presence or missing of antibodies one can not conclude a success or non-success of a therapy.
3. On the basis of several independent studies, it has been shown that FHA has cross-reactivity with Bordetella pertussis, B. parapertussis and other pathogens (3, 12, 13, 21).
4. In rare cases patient sera may show ñinverseò bands (dark background, white bands), these are not to be interpreted, means the immunoblot is in those cases not interpretable. The serum should be checked using other serological methods.

11. Performance Data

11.1 Sensitivity and Specificity

A pertussis toxin ELISA was used as reference method to calculate the performance data (sensitivity and specificity for IgG and IgA) for VIROTECH B. pertussis + CatACT LINE.
264 sera were tested for IgG. Sera: blood donors (n=78), vaccination sera (n=14), sera with suspected Bordetella pertussis Iinfections (n=119), Mycoplasma pneumoniae sera (n=24), and cross-reactive sera (n=24). 5 tested sera could not be assigned to any group.

IgG

(n=264)		B. pertussis + CatACT LINE IgG		
		Negative	Borderline	Positive
Pertussis Toxin ELISA	Negative	**144**	**13**	**1**
	Borderline	**4**	**3**	**1**
	Positive	**3**	**8**	**87**

Borderline results are excluded from the calculation.

The sensitivity for IgG was calculated from these findings to be 96.7 %, with a specificity of 99.3 %.

272 sera were tested for IgA. Sera: blood donors (n=82), vaccination sera (n=14), sera with suspected Bordetella pertussis infections (n=121), Mycoplasma sera (n=27), and cross-reactive sera (n=24). 4 tested sera could not be assigned to any group.
IgA

(n=272)		B. pertussis + CatACT LINE IgA		
		Negative	Borderline	Positive
Pertussis Toxin ELISA	Negative	**204**	**5**	**6**
	Borderline	**3**	**2**	**5**
	Positive	**6**	**3**	**38**

Borderline results are excluded from the calculation.

The sensitivity for IgA was calculated from these findings to be 86.4 %, with a specificity of 97.1 %.

11.2 Cross-Reactivity

For the examination of possible cross-reactions of the VIROTECH LINE B. pertussis + CatACT IgG/IgA with respiratory diseases, 109 (IgG) respectively 111 (IgA) sera with a suspicion towards a Mycoplasma or Chlamydia infection have been tested.

	IgG	IgA
negative	91	106
borderline	10	2
positive	8	3

The VIROTECH B. pertussis + CatACT LINE is a very good tool for a differential-diagnostic usage.

11.3 Prevalence (Expected values)

The cut-off adjusting has been chosen in a way that ideally only acute infections and those with a high vaccination titre are detected. The following table shows the results of 80 (IgG) respectively 84 (IgA) bloodbank sera:

	IgG	IgA
negative	71	78
borderline	6	2
positive	3	0

11.4 Intra-Assay-Precision (Repeatability)

At every release of a batch, one strip of each Immunoblot is tested in IgG and IgA with a specific serum in the Quality Control Dept. This means a 100% Quality Control of all Immunoblots is done.
The intensities of the bands may deviate maximum one intensity level from the mean value on a scale of 1-5.

11.5 Inter-Assay-Precision (Reproducibility)

The detection of the test precision has been effected in 10 independent test runs. Different persons were testing manually and on automates.

A negative serum, a borderline serum and a positive serum have been tested on the IgG and IgA LINE each.

	IgG
negative	10
borderline	10
positive	10

	IgA
negative	10
low positive	8 / (2*)
positive	10

(*) The low positive IgA serum has been assessed borderline twice.

11.6 Vaccination Antibodies

Since a few years acellular vaccines are used for most of the Bordetella vaccination programmes. Those contain basically the ingredients FHA and Pertussis-Toxin. In the IgG immune response to vaccination, it can be observed in many vaccinated subjects that antibodies against FHA and PT are formed but soon disappear once again. Even as little as 1 year later the titre usually corresponds once again to the level before vaccination (18).

It is repeatedly described that the IgA immune response is nearly not found in infants and does only develop after an infection (1)

12. Additional performance data for the CatACT band in IgG

12.1 Sensitivity and specificity of the CatACT band in IgG

Sensitivity

34 sera from Bordetella reference centers were tested for the B.pertussis + CatACT LINE in IgG. 23 of these sera gave a positive CatACT Band. This corresponds to a sensitivity of 67.6%.

Specificty

Blood donor sera (n=76) and vaccination sera (n=14) were tested on the B.pertussis + CatACT LINE in IgG. 12 of these sera gave a positive CatACT Band. This corresponds to a specificity of 86.2%.

Since IgA antibodies are poorly reliable markers for Bordetella sp. infections, no sensitivity and specificity of the CatACT band in IgA is indicated.

13. Additional performance data for Bordetella parapertussis in IgG and IgA

13.1 Sensitivity and specificity for Bordetella parapertussis

Sensitivity

Among 136 sera suspected of B. pertussis infection, the proportion of sera positive for *B. parapertussis* was 5.1% in IgG and 6.6% in IgA. This corresponds to the values in the literature (19, 20).

Specificity

79 blood donors were tested in IgG and IgA. Specificities of 97.2% and 100.0% respectively were achieved.

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15. Test Procedure Scheme

Test Procedure in short version

Samples Incubation	**30 minutes**	15 µl Patient serum/ plasma 100 µl control in 1,5 ml dilution-/washbuffer each
Washing	**3 x 5 minutes**	with 1,5 ml dilution-/washbuffer each
Conjugate incubation	**30 minutes**	with 1,5 ml working dilution (1 + 100)
Washing	**3 x 5 minutes** **1 x 1 minute**	with 1,5 ml dilution-/washbuffer each with Aqua dest./deionised
Substrate incubation	**10 ± 3 minutes**	with 1,5 ml ready to use substrate solution each
Stopping	**3 x without incubation in between**	with 1,5 ml Aqua dest./deionised each

Conjugate Dilution table (rounded)

Number of strips	1	2	3	4	5	6	7	8	9	10
Dilution-/washbuffer	**1,5ml**	**3,0ml**	**4,5ml**	**6,0ml**	**7,5ml**	**9,0ml**	**11,0m**	**12,0m**	**14,0m**	**15,0m**
Conjugate-concentrate	**15µl**	**30µl**	**45µl**	**60µl**	**75µl**	**90µl**	**110µl**	**120µl**	**140µl**	**150µl**
Final volume	**1,515**	**3,03m**	**4,545**	**6,06m**	**7,575**	**9,09m**	**11,11**	**12,12**	**14,14**	**15,15**

Number of strips	11	12	13	14	15	16	17	18	19	20
Dilution-/washbuffer	**17,0m**	**18,0m**	**20,0m**	**21,0m**	**23,0m**	**24,0m**	**26,0m**	**27,0m**	**29,0m**	**30,0m**
Conjugate-concentrate	**170µl**	**180µl**	**200µl**	**210µl**	**230µl**	**240µl**	**260µl**	**270µl**	**290µl**	**300µl**
Final volume	**17,17**	**18,18**	**20,2m**	**21,21**	**23,23**	**24,24**	**26,26**	**27,27**	**29,29**	**30,3m**

Number of strips	21	22	23	24	25	26	27	28	29	30
Dilution-/washbuffer	**32,0m**	**33,0m**	**35,0m**	**36,0m**	**38,0m**	**39,0m**	**41,0m**	**42,0m**	**44,0m**	**45,0m**
Conjugate-concentrate	**320µl**	**330µl**	**350µl**	**360µl**	**380µl**	**390µl**	**410µl**	**420µl**	**440µl**	**450µl**
Final volume	**32,32**	**33,33**	**35,35**	**36,36**	**38,38**	**39,39**	**41,41**	**42,42**	**44,44**	**45,45**

Number of strips	31	32	33	34	35	36	37	38	39	40
Dilution-/washbuffer	**47,0m**	**48,0m**	**50,0m**	**51,0m**	**53,0m**	**54,0m**	**56,0m**	**57,0m**	**59,0m**	**60,0m**
Conjugate-concentrate	**470µl**	**480µl**	**500µl**	**510µl**	**530µl**	**540µl**	**560µl**	**570µl**	**590µl**	**600µl**
Final volume	**47,47**	**48,48**	**50,5m**	**51,51**	**53,53**	**54,54**	**56,56**	**57,57**	**59,59**	**60,6m**