Mycoplasma pneumoniae LINE

IgG / IgA / IgM Line Immunoblot

Order No.:
WE214A16: IgA Line Immunoblot, 16 strips
WE214G16: IgG Line Immunoblot, 16 strips
WE214M16: IgM Line Immunoblot, 16 strips

FOR IN-VITRO DIAGNOSTIC ONLY

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Contents

1. Intended Use

Line Immunoblot Testkit for the quantitative detection of specific IgG, IgA and IgM antibodies in human serum. Line Immunoblot is used for the serological diagnostic testing of a fresh or recent *Mycoplasma pneumonia* infection. The kit can be used for serological diagnostic testing alone. Alternatively, it can be used as a confirmatory test, if the result of another assay is questionable or positive. The LINE has not yet been evaluated for specific questions, such as the differentiated identification of pathogens in post-infectious arthritis or in the Guillain-Barree Syndrome.

2. Diagnostic Relevance

The bacteria *Mycoplasma pneumoniae*, which is lacking cell wall components, is the cause of atypical pneumonia and tracheobronchitis of humans and affects mostly children, young adults and immunodeficient people (1,2,3,4). So called adhesins (6), enable the bacteria to attach to the epithelial cells, against which the host develops antibodies. Studies made by Foy show, that in the USA 15 to 20% of all pneumonia cases are caused by *Mycoplasma pneumoniae* (7). The infection is endemic with small epidemic peaks appearing all 4-5 years (7, 10). Mycoplasma pneumoniae is weakly infectious and transmitted only after close contact (10). Studies have shown that Mycoplasma infections are not rare in AIDS patients (8). A past infection is no protection against a re-infection (11).

The incubation time during an infection with *Mycoplasma pneumoniae* is 10 – 21 days:

- Specific IgM-antibodies occur 6-10 days after infection. Basically, about 80% of the patients younger than 20 years develop IgM-antibodies and 40% of the patients that are older than 20 years. This means a specific IgM-response can be missing especially in older patients. IgM-antibodies may be detected, referring to literature, still at least one year after beginning of the symptoms.
- Specific IgG-antibodies appear 9-14 days after infection.
- Specific IgA-antibodies appear one week after start of the infection and decrease about 5 weeks after start of the infection again. As a rule, the IgA-titer exceeds, as a rule, the IgM-titer.

Considering the fact that IgM-antibodies persist very long in some persons and are missing in others completely, it is important to detct beside the IgM- also the specific IgG- and IgA-titer. Re-infections often take place without any production of IgM-antibodies but under significant increase of IgG- and IgA-antibody titers. Two patient sera, taken at an interval of 5-10 days allow a proper statement concerning the rise of the antibody titer (5). It is important to consider that a first attack of Mycoplsma pneumoniae does not leave a sufficient protection against a new colonization. For diagnosis it is necessary in any case to consider the clinical picture in addition to the serological results.

Mycoplasma infections are generally treated successfully with antibiotics like Tetracycline and Macrolide. The treatment with non-suitable, w.g. cell-wall-specific antibiotics (penicillin) leads to a serological advantage for Mycoplasma against all Penicillin-sensitive microorganisms. Thus, a fast and specific laboratory diagnosis of this infection is very important for the beginning of a suitable therapy.
In a comparative overview of mycoplasma diagnostic testing in 2003, the then current Virotech Mycoplasma pneumoniae Western Blot was described as possessing the highest specificity of any commercially available method (9). The Mycoplasma pneumoniae LINE is an improved follow-up version of this Western Blot product.

3. Principle of Test

Pathogen antigen proteins are transferred onto a nitrocellulose membrane by a special spraying process. The nitrocellulose membrane is then cut up into individual 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-, IgA-, respectively IgM-antibodies. After unbound conjugated antibodies have been removed by a further washing step, a visualisation of the antigen/antibody-complex (of the bound antibodies) is accomplished by the addition of a non-coloured substrate, which forms blue-violette precipitates at each site („antigen bands“) 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 interpret the presence of specific IgG-, IgA-, respectively IgM-antibodies.

4. Package Contents

4.1 Kit for 16 determinations

1.	**IgG resp. IgM or IgA Nitrocellulose test strips** with sprayed antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use	**1x**	16 strips
2.	**IgG resp. IgM or IgA Cut off Control,** human serum, prediluted	**1x**	0,5ml
3.	**Dilution-/ washbuffer**, pH 7.3 (10x conc.), with Tris and preservative	**1x**	50 ml
4.	**IgG- resp. IgM or 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
	Evaluation Record sheet for the notation and deposit of the results	**1x**	1 pcs.

Also available on request:

IgG resp. IgM 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: WE214P60 resp. IgA: WE214P40 or IgM: WE214P80)

IgG/IgM/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/IgM/IgA: WE214N50)

5. Storage and Stability of the Testkits and the Components

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.
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 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. Additional 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. For CSF samples, please refer to the separate instructions for the CSF LINE.

9. Test Procedure

Working exactly according to the Sekisui Virotech user manual is the prerequisite for obtaining correct results.

9.1 Preparation of the Samples

1. 15 µl serum or plasma are needed for each patient sample. For **CSF/serum processing**, use only the separate individually calculated CSF / serum dilution for each IgG class (see instructions for the CSF LINE).
2. Blood samples should be taken separately by venous puncture. Serum is separated after complete coagulation (not applicable to plasma).
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 1000 x g), pipette clear supernatant and use for testing.

9.2 Preparation of Reagents

1. To facilitate routine laboratory work, all LINEs and EcoBlots 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 A distilled or deionised water), mix well. The dilution-/wash buffer, concentrated or already diluted, may eventually show a yellow dye. This yellow dye has no influence to the shelf life of the dilution-/wash buffer nor does it influence the functionality or diagnostic meaning of the test run.

5. **IgG-, IgA-, resp. IgM 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: The nitrocellulose test strips may only be tested in the approved Ig class (see label on blot booklet and indication on each individual test strip).

For the correct performance and evaluation of the Mycoplasma pneumoniae LINEs, each test run should include the appropriate parameter and batch-specific cut off controls.

For reliable diagnostic testing for *Mycoplasma pneumoniae*, the LINE should be performed in IgG, IgA and IgM.

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 the patient serum and control for **30 minutes** on the rocking platform. Ensure that no cross-contamination occurs between individual patient samples during pipetting and subsequent pouring away.
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 portions of ready-to-use **substrate solution** into the channels and develop for **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 enclosed evaluation record sheet for evaluation. The high specificity bands annotated on the record sheet facilitate evaluation of the patient samples.

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 strip 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 LINE strip is fitted out with a conjugate control band which appears after incubation with the respective conjugate.

The test procedure is valid, if the serum control as well as the internal conjugate control appears clearly visible on the developed nitrocellulose test strip.

The position of the serum band and conjugate control band may be found on the record sheet.

10.1 Evaluation of the patient samples

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

IgG bands: P1, P90, P400, NMP, RP3M, RP3F and P1-EPI

IgA bands: P1, P90, P400, RP14, P200

IgM bands: P1, P90, P400, Pdh-B, GL, I-Prot.

10.2 Use of the cut-off control

Bands which are weaker than the cut-off band (P1) of the cut-off control are not included in the evaluation. The P1 band must be of low intensity.

Evaluation of the band intensities (consider exceptions: Pdh-B, GL, I-Protein, RP3M, RP3F and P1-EPI):

P1 band: The evaluation of all protein bands in the IgG, IgA and IgM is related to the intensity of the P1 band of the cut-off control as follows:

- **Lower intensity than the P1 band of the cut-off control = 0**
- **Same intensity as the P1 band of the cut-off control = 1**
- **Greater intensity than the P1 band of the cut-off control = 2**

The sum of the band intensities gives the overall evaluation.

Important exceptions:

- In the IgM, the bands **Pdh-B, GL and I-Protein** are only evaluated if at least one of the bands P1, P90 or P400 is ≥ the cut-off band, i.e. it is evaluated with 1 or 2.
- In the IgG, only one of the bands **RP3M and RP3F** is evaluated. The more strongly reactive band is used for the evaluation.
- In the IgG, the **seroprevalence band P1-EPI**, is not included in the sum. This is evaluated as positive when its intensity is ≥ P1 band of the cut-off control. If the overall evaluation in all IgG classes is also negative, this indicates that the patient had contact with Mycoplasma pneumoniae in the distant past.

10.3 Significance of the antigens

List of the recombinants used (P1, P90, P400, RP3M, RP3F, RP14, P200) and purified native antigens (NMP, Pdh-B, GL, I-Protein)

Antigen / Designation	Significance of the Antigens	Specificity of the Antibodies in the LINE

P1	Protein P1 is the main adhesin (main antigen) of *M. pneumoniae* (Mw 176 kDa). It is expressed on the surface, localised in the Tip region and responsible for cytoadherence.	Highly specific
P90	P90 is expressed on the surface and is responsible for the correct and specific integration of the P1 protein into the bacterial membrane.	Highly specific
P400	The function of P400 is largely unknown.	Specific
NMP	Low molecular weight proteins. Membrane components and surface expressed proteins.	Specific
RP3M & RP3F	On the basis of sequence differences in gene P1, isolates of *M. pneumoniae* are assigned to serotype 1 - M129 (RP3M) - or to serotype 2 - FH (RP3F).	Highly specific
RP14	RP14 is the rec. C-terminal section of the P1 adhesin. Antibodies to RP14 can inhibit the cytoadherence of *M. pneumoniae* to HBEC (human bronchial epithelial cells).	Highly specific
P200	P200 is involved as a structural protein in the formation of the cytoskeleton of *M. pneumoniae*. It permits the bacterium to slide on surfaces, so that successful host colonisation is then possible.	Highly specific
Pdh-B	Pdh-B is a component of pyruvate dehydrogenase. Pdh-B is expressed on the surface and is one of the five most frequent proteins (by weight) in *M. pneumoniae*.	Possible acute marker in combination with highly specific M. pn. antigens
GL	*M. pneumoniae* is only coated with a double layer membrane, surrounded by a lipoglycan layer. In this context, it is to be expected that phospholipids and glycolipids – essential components of membranes – will be, to some extent, presented on the cell surface of the bacterium, where they are recognised by the human immune system.	Possible acute marker in combination with highly specific M. pn. antigens
I-Protein	I-Proteins are erythrocyte antigens, which are recognised by cold agglutinins (CA). The CAs are induced by *M. pneumoniae* and are of IgM type and are directed against I-protein in more than 90% of cases.	Possible acute marker in combination with highly specific M. pn. antigens
P1-EPI	A mixture of the P1 antigens of strains FH and M129, and shows the seroprevalence in the IgG.	Highly specific

10.4 Evaluation Criteria

The interpretation of serological results should always incorporate the clinical picture epidemiological data and other available laboratory findings.

IgG or IgA Evaluation	
Sum of the Band Intensities	**Evaluation**
< 4	Negative
= 4	Borderline
> 4	Positive

IgM Evaluation	
Sum of the Band Intensities	**Evaluation**
< 3	Negative
= 3	Borderline
> 3	Positive

10.5 Interpretation Scheme IgG, IgA and IgM

Evaluation	Interpretation	
Negative	No serological evidence for *Mycoplasma pneumoniae* infection or status after an infection in the distant past	A positive seroprevalence band P1-EPI in the IgG (≥ cut-off band) indicates earlier contact with *Mycoplasma pneumoniae.*
Borderline	Antibodies against *Mycoplasma pneumoniae* are detectable. Weaker reaction during convalescence, with persistent antibodies or in the initial stages of an infection. A follow-up is recommended.	
Positive	Antibodies to *Mycoplasma pneumoniae* are detectable. Indicates fresh or recent infection with *Mycoplasma pneumoniae*.	

10.6 Overall Constellations of Findings (IgG, IgA and IgM)

IgG	IgA	IgM	Interpretation
-	-	-	No contact with *Mycoplasma pneumoniae* or antibody levels have already dropped to below the cut-off level.
-	+	+	Very early phase of an acute infection or re-infection.
-	+	-	Very early phase of an acute infection; either first infection or re-infection without IgM or IgM titre has not yet increased.
+	+	+	Acute infection, usually first infection, late phase, IgG and IgM already formed, IgA has not yet decreased.
+	-	+	Acute infection, usually first infection, late phase, IgG and IgM already formed, IgA has already decreased.
+	+	-	Re-infection, very late phase, IgA still present, no more IgM present, or re-activation or infection without IgM formation
+	-	-	Re-infection, very late phase, IgA has already decreased or was never formed (happens with some adults) or re-activation or infection without formation of IgM or persistent IgG titre after completion of an infection
-	-	+	Acute early infection, IgA still missing or has already decreased, IgG titre still too low.

10.7 Test Limits

1. A negative Blot result does not completely exclude the possibility of infection with *Mycoplasma pneumoniae*. The sample may have been taken before antibodies developed or the antibody concentration is under the limit of detection of the test.
2. In rare cases, patients may exhibit “inverse” bands (dark background, white bands); these should not be evaluated, i.e. the Immunoblot is not evaluable in these cases. The serum should be tested with other serological methods.

11. Performance Data

11.1 Analytical Sensitivity and Specificity

To determine the analytical sensitivity and specificity, groups of sera were tested in the IgG, IgA and IgM, which had previously been determined with an ELISA and a Western Blot as reference method (analytical finding).
The following groups of sera were tested: blood donors (n=52), cross-reactors (n=69), children’s sera (n=27), mycoplasma sera (n=52).

IgG

Serum Group (n=200)		Mycoplasma pneumoniae LINE IgG		
		Negative	Borderline	Positive
Analytical	Negative	125	12	2

Finding	Borderline	10	1	3
	Positive	3	4	40

For the IgG, this gives a sensitivity of 93.0% and a specificity of 98.4%.
Borderline results are excluded from the calculation.

IgA

Serum Group (n=200)		**Mycoplasma pneumoniae LINE IgA**		
		Negative	Borderline	Positive
Analytical Finding	Negative	138	10	11
	Borderline	10	5	5
	Positive	0	2	19

For the IgA, this gives a sensitivity of >99% and a specificity of 92.6%.
Borderline results are excluded from the calculation.

IgM

Serum Group (n=200)		**Mycoplasma pneumoniae LINE IgM**		
		Negative	Borderline	Positive
Analytical Finding	Negative	126	7	8
	Borderline	4	1	8
	Positive	1	2	43

For the IgM, this gives a sensitivity of 97.7% and a specificity of 94.0%.
Borderline results are excluded from the calculation.

11.2 Seroprevalence (expected values)

The cut-off setting was performed in such a way that fresh or recent Mycoplasma pneumoniae infections were detected. The following table shows the results from 52 blood donor sera:

	IgG	**IgA**	**IgM**
Negative	49	46	48
Borderline	3	2	2
Positive	0	4	2

Seroprevalence band P1-EPI in the IgG

Of 148 sera (blood donor sera n=52, cross-reactive sera n=69 and children's sera n=27), 89 exhibited a P1-EPI band > cut off (=60.1%).

11.3 Intra-Assay Precision (repeatability)

At each batch release, a strip with a specific human serum was tested in the IgG, IgA and IgM in the quality control. Thus 100% of all Immunoblots were controlled.
The intensities of the bands may deviate from the mean by maximally one step on a 1-5 point scale.

11.4 Inter-Assay Precision (reproducibility)

To determine the reproducibility, 4 sera each were tested in the IgG, IgA and IgM. The determination was performed in 10 test batches on 6 independent test days.
The serological requirements were fulfilled in all tests.

12. Literature

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13. 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 minutes**	with 1,5 ml dilution-/washbuffer each with Aqua dest./deionised
Substrate incubation	**10 ± 3 minutes**	with 1,5 ml 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	11,0ml	12,0ml	14,0ml	15,0ml
Conjugate-concentrate	15µl	30µl	45µl	60µl	75µl	90µl	110µl	120µl	140µl	150µl
Final volume	1,515ml	3,03ml	4,545ml	6,06ml	7,575ml	9,09ml	11,11ml	12,12ml	14,14ml	15,15ml

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

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

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