VIROTECH Influenza A IgG/IgM ELISA
(Influenza A IgG/IgM ELISA)

Order No.: EC118.00

VIROTECH Influenza A IgA ELISA
(Influenza A IgA ELISA)

Order No.: EC118A00

VIROTECH Influenza B IgG/IgM ELISA
(Influenza B IgG/IgM ELISA)

Order No.: EC119.00

VIROTECH Influenza B IgA ELISA
(Influenza B IgA ELISA)

Order No.: EC119A00

Color Coding:	**Influenza A:**	**IgG/IgM:**	**light-blue**
		IgA:	**light-blue / black**
	Influenza B:	**IgG/IgM:**	**light-blue / transparent**
		IgA:	**light-blue / red**

FOR IN VITRO DIAGNOSIS ONLY

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REV 36 / VIROTECH Influenza A & B ELISA IgG/IgM/IgA EN

Contents

1. Intended Use

The influenza ELISA tests are used to detect human influenza A or influenza B virus antibodies in serum. To detect vaccine antibodies and fresh infections, native core antigens and also recombinant haemagglutinins (HA) are used. The recombinant haemagglutinins are updated annually. (Influenza A: A/Guangdong-Maonan/SWL1536/2019 (H1N1) -like virus, A/Hawaii/70/2019/ (H1N1)-like virus; Influenza B: B/Washington/02/2019-like virus).

2. Diagnostic Relevance

The cause of influenza A/B (viral flu) is one of the orthomyxoviruses. Extremely high genetic variability is characteristic of influenza viruses, based on an above-average mutation frequency and the capacity for gene exchange. These features lead to the epidemics and endemics typical of influenza.

Influenza virus infections are distributed worldwide, and besides humans, birds and mammals represent another natural reservoir for Influenza A.

Because of the great genetic variability of the influenza virus, the composition of the influenza vaccine must be regularly updated. Reference laboratories throughout the world continuously analyse the circulating influenza viruses and communicate their data to the World Health Organization (WHO). On the basis of these data, the WHO annually publishes a vaccine recommendation for the southern and northern hemispheres. For the northern hemisphere and the 2018/2019 season, the recommendation for three-component vaccines is the following: Influenza A: A/Guangdong-Maonan/SWL1536/2019 (H1N1)-like virus, A/Haweii/70/2019/ (H1N1)-like virus; Influenza B: B/Washington/02/2019-like virus (4).

Flu (influenza) is an acute respiratory tract infection. Spread is airborne and it is highly contagious. The incubation period is 1-3 days. In the northern hemisphere, flu occurs mainly between December and April, but sporadic cases are observed throughout the year.

The early diagnosis of an influenza infection is best made by isolating the virus (culture/ PCR/ EIA/ IFT) from nasal and throat swabs (2). Culturing the virus followed by immunohistochemical detection using monoclonal antibodies is achieved as gold standard for direct detection of Antigens.

In serological diagnosis, ELISA and CBR are used to find type-specific antibodies (2) and to differentiate the individual immunoglobulin classes. The measurement of subtype-specific antibodies is unimportant in clinical diagnosis because of the high variability and an infection rate of 50 % (3). An IgG antibody titre can only indicate contact with the antigen, but this does not necessarily mean immunity. This also applies for proven vaccination titres (1). Serology is only of limited use to diagnose an acute event, as the antibodies generally occur only 2-3 weeks after the onset of the disease (2). They are of increased significance in epidemiological surveys.

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/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. **IgG negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
5. **IgG cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgG positive Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
7. **IgM negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
8. **IgM cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
9. **IgM positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
10. **IgG-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use

11. **IgM-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
12. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
13. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

4.2 IgA Testkit

14. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
15. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7,2, with preservative and Tween 20
16. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
17. **IgA negative Control, 2000µl,** human serum with protein-stabilizer and preservative, ready to use
18. **IgA cut-off Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
19. **IgA positive Control, 2000µl,** human serum with protein-stabilizer and preservative, ready to use
20. **IgA-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
21. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
22. **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), IgG- IgM- and IgA-positive, negative and cut-off controls 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 IgG-, IgA- and 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-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.

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

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

9.3 Interpretation of Results

With Influenza IgG antibodies occur about 2-3 weeks after infection (2). Therefore a positive result may give notice of an acute or recent infection (please note vaccination management!). However these results should always be considered in the context of all available medical findings. A final diagnosis is only possible in consideration with anamnesis, clinic and laboratory data. The appearance of increased IgA or IgM titers may give additional notice of an acute infection. They might also occur in reinfections and may persist up to one year.

It is advantageous to recognize titer changes in the Ig-classes to make Influenza diagnostic more secure. The examination of titer courses (first serum shortly after infection, second serum after 14 days) may serve as help when interpretating unclear diagnosis.

9.4 Interpretation Scheme IgG, IgM and IgA

Result (VE)	Evaluation	Interpretation
< 9,0	Negative	Antibodies without significant concentration
9,0 - 11,0	Borderline	No significantly increased antibody concentration repeat test, if necessary obtain 2nd sample
> 11,0	Positive	Significantly increased antibody concentration • Notice of acute infection • Notice of past infection • Vaccination antibodies

9.5 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.
2. No sera from patients with acute Influenza infection have been available for evaluation. Therefore the performance data are based upon tests of vaccination sera and blood bank sera.
3. Crossreactivities between Influenza A and Influenza B may occur.
4. The RKI recommends that the diagnostic laboratory test should be repeated within a short interval if there are still clinical signs of the disease.
5. The use of the current recombinant HA antigens has not changed the performance data. It is possible that the use of these antigens in some sera may lead to an improvement in diagnosis.

10. Performance Data

10.1 Sensitivity and Specificity

Diagnostic performance was determined using a serum panel of 112 sera. These were tested in the VIROTECH Influenza A IgG/IgM/IgA ELISA and in the ELISA of a competitor.

Influenza A IgG

n = 81		Competitor	
		Pos	Neg
VIROTECH	Pos	68	0
	Neg	2	11

PPA: 97%
NPA: 100%

The diagnostic sensitivity is 97%, the diagnostic specificity 100%. Sera assessed as borderline were not considered in the calculation of the performance parameters. In the competitor's test 24 of 112 (21%) samples showed a borderline result, in the VIROTECH test 13 of 112 (12%) samples showed a borderline result.

Influenza A IgM

n = 89		competitor	
		Pos	Neg
VIROTECH	Pos	28	3
	Neg	0	58

PPA: 100%
NPA: 95%

The diagnostic sensitivity is 97%, the diagnostic specificity 95%. Sera assessed as borderline were not considered in the calculation of the performance parameters. In the competitor's test 11 of 112 (10%) samples showed a borderline result, in the VIROTECH test 15 of 112 (13%) samples showed a borderline result.

Influenza A IgA

n = 87		competitor	
		Pos	Neg
VIROTECH	Pos	29	3
	Neg	5	50

PPA: 85%
NPA: 94%

The diagnostic sensitivity is 85%, the diagnostic specificity 94%. Sera assessed as borderline were not considered in the calculation of the performance parameters. In the competitor's test 13 of 112 (12%) samples showed a borderline result, in the VIROTECH test 14 of 112 (13%) samples showed a borderline result.

Influenza B ELISA

To determine the sensitivity and specificity sera were tested in the VIROTECH ELISA and in an ELISA of a competitor.

- 83 sera were tested in IgG. The calculated sensitivity is 77%, the calculated specificity is >99.9%.
- 88 sera were tested in IgM. The calculated sensitivity is 89%, the calculated specificity is >99.9%.
- 85 sera were tested in IgA. The calculated specificity is 89%. Owing to a lack of positive sera, the sensitivity for IgA could not be calculated.

10.2 Prevalence (Expected Values)

Influenza A ELISA

78 sera of healthy blood donors were tested in VIROTECH Influenza A IgG/IgM/IgA ELISA.

n = 78	**IgG**	**IgM**	**IgA**
negative	30	67	66
borderline	24	6	5
positive	24	5	7

The prevalence of Influenza A-specific IgG antibodies in healthy blood donors is 61.5%, where borderline sera were found to be positive. This result is very close to the 50% rate of infection described in the literature (3).

To determine the level of Influenza A specific IgG antibodies in vaccinated individuals, 46 sera from vaccinated individuals were tested in the Influenza A IgG ELISA. The prevalence of influenza A specific IgG antibodies in vaccinated individuals is 100%, with borderline sera (1 in 46) being positive.

Influenza B ELISA

The following tables show the results of the examination of blood bank sera:

	IgG n=78		IgM n=78		IgA n=78	
	No.	**%**	**No.**	**%**	**No.**	**%**
Negative	47	60	75	96	75	96
Borderline	11	14	0	0	3	4
Positive	20	26	3	4	0	0

10.3 Intra-assay-Coefficient of Variation (Repeatability) and

10.4 Inter-assay-Coefficient of Variation (Reproducibility)

Influenza A ELISA

A precision sample panel consisting of samples that completely cover the diagnostically relevant measuring range (1x 2-6 VE, 1x 6-11 VE, 1x 9-14 VE and 1x 14-25 VE) in a total of 10 independent runs over a period of 4 days. The tests were performed individually by three different persons. Repeatability (intra-assay coefficient of variation) and reproducibility (inter-assay coefficient of variation) were calculated.

Repeatability: Within the diagnostically significant test area around the cut off, the repeatability for all IgX classes is below 8%.

Reproducibility: Within the diagnostically significant test area around the cut off, the reproducibility for all IgX classes is below 11%.

VIROTECH Influenza A IgG ELISA

		repeatability		reproducibility	
	mean value VE	**SD**	**% VK**	**SD**	**% VK**
Sample 1	5,8	0,2	3,6	0,3	5,2
Sample 2	8,7	0,3	3,0	0,5	5,5
Sample 3	12,5	0,3	2,6	0,8	6,4
Sample 4	17,3	0,4	2,2	1,1	6,5

VIROTECH Influenza A IgM ELISA

		repeatability		reproducibility	
	mean value VE	**SD**	**% VK**	**SD**	**% VK**
Sample 1	5,4	0,2	4,2	0,3	4,9
Sample 2	9,4	0,3	3,7	0,7	7,3
Sample 3	13,0	0,4	3,1	0,8	6,3
Sample 4	18,5	0,8	4,1	1,2	6,4

VIROTECH Influenza A IgA ELISA

		repeatability		reproducibility	
	mean value VE	**SD**	**% VK**	**SD**	**% VK**
Sample 1	3,6	0,3	7,1	0,4	10,1
Sample 2	8,4	0,3	3,3	0,8	9,5

Sample 3	9,5	0,4	4,6	0,5	5,6
Sample 4	21,4	0,7	3,0	1,2	5,8

Influenza B ELISA

In one assay, strips of different plates were tested in a chessboard pattern with two sera. The obtained coefficients of variation for Influenza B IgG, IgM, IgA are < 9%.

Four sera were tested in 10 independent test runs by different persons in different laboratories. The obtained variation coefficient values for Influenza B are <15%.

11. Literature

1. Epidemiologisches Bulletin, Nr.17.2003
2. Labor und Diagnose, L.Thomas, 5.Auflage, 1998
3. Mikrobiologische Diagnostik und Krankenhaushygiene, Max v. Pettenkofer-Institut, 2. Ausgabe 2003
4. Recommended composition of influenza virus vaccines for use in the 2020 - 2021 northern hemisphere influenza season; February 2020

Preparation of Patient Samples and Washing Solution

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

▼ IgG-/IgA-samples – Dilution 1:101

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

▼ 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** IgG, IgM, IgA
⇩		
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)**