Product information

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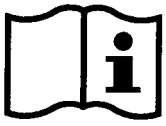

Useŕs Manual

Influenza A IgG ELISA

Enzyme immunoassay for the detection and quantitative determination of human IgG antibodies against Influenza A in serum and plasma

IVD **For *in vitro* diagnostic use only.**

REF **IB79251**

96 wells

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955 Fax.: +1 (763) - 780-2988
Email: info@ibl-america.com Web: www.ibl-america.com

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Contents

1. INTENDED USE

The IBL-AMERICA Influenza A IgG Antibody ELISA Test Kit has been designed for the detection and quantitative determination of specific IgG antibodies against Influenza A in serum and plasma. For *in vitro* diagnostic use only.

2. GENERAL INFORMATION

Influenza infection is an acute feverish virus infection, which principally leads to an illness of the respiratory tract and appears as an epidemic or pandemic. The infection mostly spreads via droplet infection. The virus spreads from the mucous membrane of the upper respiratory to the whole bronchial tract. There the virus and its toxin can lead to a serious inflammation of the bronchial mucosa and damage of the vessels. After an incubation time of 1 to 3 days symptoms appear suddenly: Followed by elevated temperature, often accompanied by shivering, the catarrhal leading symptom appears, and common symptoms include painful dry cough, tracheitis, laryngitis and frequently a rhinitis and conjunctivitis.
The Influenza viruses form a virus group with principally similar morphological, chemical and biological features. The types A, B and C were defined, from which many other variants are known. The distinction of the types can be made through the different antigenicity of their nucleoproteins, which are coated by a matrix protein with type-specific antigenicity, too. However, both internal antigens are of less importance for immunity. The essential antigens are the hemagglutinin and the neuraminidase. Both are surface antigens and subject to a permanent change of their antigenicity, which is called drift or shift. The appearance of permanent new Influenza epidemics and pandemics are particularly facilitated by an antigen variability, because the new drift or shift variants infect a population which is only partly immune or in an extreme case completely susceptible to the disease.

3. PRINCIPLE OF THE TEST

The IBL-AMERICA Influenza A IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Influenza A antigen is bound on the surface of the microtiter strips. Diluted serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Influenza A antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

4. LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

5. REAGENTS PROVIDED

Symbol	Components	Volume / Qty.
SORB MT	Influenza A antigen coated microtiter strips	12
CAL A	Calibrator A (Negative Control)	2 mL
CAL B	Calibrator B (Cut-Off Standard)	2 mL
CAL C	Calibrator C (Weak Positive Control)	2 mL
CAL D	Calibrator D (Positive Control)	2 mL
ENZ CONJ	Enzyme Conjugate	15 mL
SUB TMB	Substrate	15 mL
STOP SOLN	Stop Solution	15 mL
SAM DIL	Sample Diluent	60 mL
WASH SOLN 10x	Washing Buffer (10×)	60 mL

Storage and Stability (refer to the expiry date on the outer box label)

Store kit components at 2-8ºC and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25ºC). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8ºC. After the first opening the kit should be used within 3 months, the diluted wash buffer can be kept for 4 weeks at 2-8°C.

5.1. Microtiter Strips
12 strips with 8 breakable wells each, coated with a Influenza A antigen (strains Beijing 265/95 (H_1N_1) and Sydney 5/97 ($H3N_2$)). Ready-to-use.

5.2. Calibrator A (Negative Control)
2 mL, protein solution diluted with PBS, contains no IgG antibodies against Influenza A. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.3. Calibrator B (Cut-Off Standard)
2 mL human serum diluted with PBS, contains a low concentration of IgG antibodies against Influenza A. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.4. Calibrator C (Weak Positive Control)
2 mL, human serum diluted with PBS, contains a medium concentration of IgG antibodies against Influenza A. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.5. Calibrator D (Positive Control)
2 mL, human serum diluted with PBS, contains a high concentration of IgG antibodies against Influenza A. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.6. Enzyme Conjugate
15 mL, anti-human-IgG-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.7. Substrate

15 mL, TMB (tetramethylbenzidine). Ready-to-use.

5.8. Stop Solution
15 mL, 1 N acidic solution. Ready-to-use.

5.9. Sample Diluent
60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

5.10. Washing Buffer
60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

6. MATERIALS REQUIRED BUT NOT PROVIDED

- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter Plate Reader (450 nm)
- Microtiter Plate Washer
- Reagent tubes for the serum dilution
- Bi-distilled water
- Plastic Bag

7. SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

8. ASSAY PROCEDURE

8.1. Preparation of Reagents

Washing Solution: dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- Standards and samples should be assayed in duplicates.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

8.2. Assay Steps

1. Prepare a sufficient amount of microtiter wells for the standards, controls and samples in duplicate as well as for a substrate blank.
2. Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** standards and controls respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the re-usable plate cover and incubate at room temperature for 60 minutes.
4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. After removing the third repetition of wash buffer, always remove residual moisture by inverting the microtiter plate and repeatedly tapping forcefully on a paper towel.
5. Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover plate with the re-usable plate cover and incubate at room temperature for 30 minutes.
7. Empty the wells of the plate (dump or aspirate) and repeat step 4 entirely.
8. Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
9. Cover plate with the re-usable plate cover and incubate at room temperature for 20 minutes.
10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

9. EVALUATION

Example

	OD Value	Corrected OD
Substrate Blank	0.011	
Negative Control	0.027	0.016
Cut-Off Standard	0.650	0.639
Weak Positive Control	1.431	1.420
Positive Control	2.241	2.230

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently **no reference values** which have to be found in other laboratories in the same way.

9.1 Qualitative Evaluation

The calculated absorptions for the patient sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result. For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of +/- 20% around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 week, is recommended. Both samples should be measured in parallel in the same run. The positive control must show at least the double absorption compared with the cut-off standard.

9.2 Quantitative Evaluation

The ready-to-use standards and controls of the Influenza A IgG antibody kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for controls and standards in units are printed on the QC data sheet. For a quantitative evaluation the absorptions of the standards and controls are graphically drawn point-to-point against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs. As curve fit point-to-point has to be chosen. Calibrator B with its concentration of 10 U/mL serves as cut-off standard. Analogous to the qualitative evaluation a range of +/- 20% around the cut-off is defined as a grey zone. Thus results between 8 and 12 U/mL are reported as borderline.

10. ASSAY CHARACTERISTICS

Influenza A ELISA	IgG
Intra-Assay-Precision	8.5 %
Inter-Assay-Precision	6.5 %
Inter-Lot-Precision	3.8 – 6.1 %
Analytical Sensitivity	1.09 U/mL
Recovery	100 – 114 %
Linearity	79 – 114 %
Cross-Reactivity	No cross-reactivity to RSV, Adenovirus and Parainfluenza 1/2/3..
Interferences	No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL
Clinical Specificity	88 %
Clinical Sensitivity	100 %

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Manufactured for:
Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
Phone: +1 (763) - 780-2955 Fax.: +1 (763) - 780-2988
Email: info@ibl-america.com Web: www.ibl-america.com

SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità