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IVD



See external label



2°C-8°C



Σ=96 tests

REF

Cat # 5109-8

Influenza B IgM

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Enzyme immunoassay based on microtiter plate for the detection and quantitative determination of human IgM antibodies against **Influenza B** in serum and plasma

Test	Influenza B IgM ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Indirect ELISA: Antigen Coated Plate
Detection Range	Qualitative Positive; Negative control & Cut off
Sample	100 µL
Specificity	100%
Sensitivity	100%
Total Time	~110 min
Shelf Life	12 -18 Months

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

Intended Use

The Diagnostic Automation Inc. Influenza B IgM Antibody ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgM antibodies against Influenza B in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of Diagnostic Automation Inc.

This assay is intended for in-vitro diagnostic use only.

Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

General Information

The 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 results from a 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 a damage of the vessels. After an incubation time of 1 to 3 days the symptoms appear suddenly: Followed by a fast increase of temperature, often accompanied by shivering, the catarrhal leading symptom appears, which contribute to the clinical course beside 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 will be possible by 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 the 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.

The determination of the Influenza type (A, B, and C) gives both the clinician and epidemiologist important indications for further actions. Thus Influenza B often leads to a serious clinical course and an epidemic spread of the virus. Similarly, during an Influenza A epidemic, the epidemiological importance and derived measures for the protection of the individual and population primarily stand in the foreground together with the severity of the clinical symptoms.

Principle of the Test

The Diagnostic Automation Inc. Influenza B IgM antibody test kit is based on the principle of the enzyme immunoassay (EIA). Influenza B antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgM antibodies of the serum and the immobilized Influenza B 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-IgM 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 IgM antibodies is directly proportional to the intensity of the color.

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.
- No reagents from different kit lots have to be used, they should not be mixed among one another.
- 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.

Reagents Provided

Store kit components at 2-8°C 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 (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

Components	Volume / Qty.
Influenza B antigen coated microtiter strips	12
Calibrator A (Negative Control)	2 mL
Calibrator B (Cut-Off Standard)	2 mL
Calibrator C (Weak Positive Control)	2 mL
Calibrator D (Positive Control)	2 mL
Enzyme Conjugate	15 mL
Substrate	15 mL
Stop Solution	15 mL
Sample Diluent	60 mL
Washing Buffer (10×)	60 mL
Plastic foils	2
Plastic bag	1

1. Microtiter Strips

12 strips with 8 breakable wells each, coated with a Influenza B antigen (Current vaccine, strain Harbin). Ready-to-use.

2. Calibrator A (Negative Control)

2 mL, protein solution diluted with PBS, contains no IgM antibodies against Influenza B. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

3. Calibrator B (Cut-Off Standard)

2 mL human serum diluted with PBS, contains a low concentration of IgM antibodies against Influenza B. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

4. Calibrator C (Weak Positive Control)

2 mL, human serum diluted with PBS, contains a medium concentration of IgM antibodies against Influenza B. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5. Calibrator D (Positive Control)

2 mL, human serum diluted with PBS, contains a high concentration of IgM antibodies against Influenza B. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

6. Enzyme Conjugate

15 mL, anti-human-IgM-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.

7. Substrate

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

8. Stop Solution

15 mL, 0.5 M sulfuric acid. Ready-to-use.

9. Sample Diluent

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

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.

11. Plastic Foils

2 pieces to cover the microtiter strips during the incubation.

12. Plastic Bag

Resealable, for the dry storage of non-used strips.

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
- Bidistilled water

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).

Assay Procedure

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.

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 enclosed foil 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. Residuals of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
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 enclosed foil and incubate at room temperature for 30 minutes.
7. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Residuals of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
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 enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).
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.

Evaluation

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

Example

	OD Value	corrected OD	Mean OD Value
Substrate Blank	0.011		
Negative Control	0.014 / 0.014	0.003 / 0.003	0.003
Cut-Off Standard	0.479 / 0.498	0.468 / 0.487	0.478
Weak Positive Control	1.074 / 1.118	1.063 / 1.107	1.085
Positive Control	2.317 / 2.367	2.306 / 2.356	2.331

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.

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 weeks, 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.

2. Quantitative Evaluation

The ready-to-use standards and controls of the Influenza B 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 labels of the vials.

For a quantitative evaluation the absorptions of the standards and controls are graphically drawn 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.

Assay Characteristics

Influenza B ELISA	IgG	IgA	IgM
Intra-Assay-Precision	9.2 %	7.3 %	8.2 %
Inter-Assay-Precision	8.9 %	8.0 %	9.6 %
Inter-Lot-Precision	4.8 – 11.0 %	1.1 – 5.9 %	3.3 – 9.7 %
Analytical Sensitivity	1.07 U/mL	1.38 U/mL	1.19 U/mL
Recovery	89 – 107 %	94 – 106 %	71 – 92 %
Linearity	71 – 123 %	77 – 124 %	82 – 123 %
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	89 %	100 %	100 %
Clinical Sensitivity	100 %	85 %	100 %

References

1. Drescher, J., Verhagen, W. Method for determining the equilibrium constant and the concentration of influenza virus IgG antihaemagglutinin antibody molecules by use of EIA titres determined with and without guanidine hydrochloride. *J. Virol. Methods*, **47**(3): 307-19 (1994).
2. Drescher, J., Verhagen, W. Determination of the concentration of influenza virus antihaemagglutinin antibody molecules of the IgG class and of the equilibrium constant by use of enzyme immunoassay titres determined for graded epitope concentrations. *J. Virol. Methods*, **55**(2): 257-70 (1995).
3. Lupulescu, E. et al. ELISA in the rapid diagnosis of influenza using as the detecting antibodies polyclonal antinucleoprotein sera. *Bacteriol. Virusol. Parazitol. Epidemiol.*, **41**(1-2): 63-7 (1996).
4. Marcante, R. et al. Rapid diagnosis of influenza type A infection: comparison of shell-vial culture, directigen flu-A and enzyme-linked immunosorbent assay. *New Microbiol.*, **19**(2): 141-7 (1996).
5. Marinich, IG. et al. The immunoprophylaxis of influenza among elderly persons. *Zh. Mikrobiol. Epidemiol. Immunobiol.* (1997/3): 60-4.
6. Moldoveanu, Z. et al. Human immune responses to influenza virus vaccines administered by systemic or mucosal routes. *Vaccine* **13**(11): 1006-12 (1995).
7. Naikhin, AN. et al. Immuno-enzyme analysis of post-vaccination secretory immunity to influenza A and B viruses using a manufactured monoclonal immunoenzyme test system. *Vopr. Virusol.* **42**(6): 271-5 (1997).
8. Naikhin, AN. et al. Monoclonal immuno-enzyme test-system for evaluating secretory immunity to influenza A and B viruses. *Vopr. Virusol.* **42**(5): 212-6 (1997).
9. Powers, DC. et al. Neuraminidase-specific antibody responses to inactivated influenza virus vaccine in young and elderly adults. *Clin. Diagn. Lab. Immunol.* **3**(5): 511-6 (1996).

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