Poliovirus IgG ELISA

Enzyme immunoassay for the qualitative and quantitative determination of IgG antibodies against Poliovirus in human serum and plasma.

REF  RE56921

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1. Intended Use

The Poliovirus IgG ELISA has been designed for the detection and the quantitative determination of specific IgG antibodies against Polio Serotypes 1 and 3 in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of IBL Interenational.

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.

2. General Information

Polio is an infection caused by enterovirus, which occurs epidemically world-wide, and which often leads to paralysis and death. Three types of human-pathogenic poliomyelitis viruses are actually known:

- Type 1 (Brunhilde): often with severe symptoms
- Type 2 (Lansing): with milder symptoms (exterminated)
- Type 3 (Leon): rare, but with severe symptoms

On 20th September 2015 the Global Commission for the Certification of Poliomyelitis Eradication concluded, that the wild poliovirus type 2 has been eradicated worldwide.

Polioviruses mainly proliferate in the lymph nodes of the intestine, and are excreted via feces. The throat can also be infected, and the viruses then leave the body orally. After the infection, the viruses are distributed via monocytes into other lymph nodes, where they multiply. In a second viremic phase they settle in the whole organism, amongst others in the central nervous system. In more than 90% of the infections the patient does not suffer any subjective symptoms. In the remaining other cases there appear: unspecific illness with slight fever, head and throat irritations, diarrhoea, nausea, and vomiting. Very rarely the classical paralysis with affliction of muscles and cerebral nerves is seen. The reconvalescent phase can last up to two years, frequently there stay long-lasting damages. There exists no treatment of the disease, only symptomatic therapy with acetylsalicylic acid and gymnastics is possible. In many countries of the Asiatic area poliomyelitis is still endemic, but WHO has started ambitious projects to eradicate the disease. In Europe there exist cases which are imported by tourists, sometimes with lethal outcome. The diagnosis of poliomyelitis is performed by direct detection of the infectious agent in stool or throat washings during the incubation time of the virus, or by determination of antibodies in the blood. The latter is usually done by the neutralisation test, where titer differences of paired sera against the two virus types are measured separately. Only recently a Poliomyelitis IgG ELISA assay was developed in analogy to Diphtheria and Tetanus, which can detect antibodies against the two types of Polio simultaneously. This serological response can be due to a past illness or to immunity by vaccination.

3. Principle of the Tests

Poliovirus IgG ELISA is based on the principle of the enzyme immunoassay (EIA). Polio 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 IgG antibodies of the serum and the immobilized Polio 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**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Components</th>
<th>Volume / Qty.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTP</td>
<td>Microtiter Plate</td>
<td>12</td>
</tr>
<tr>
<td>ENZCONJ IgG</td>
<td>Enzyme Conjugate IgG</td>
<td>15 mL</td>
</tr>
<tr>
<td>CAL A</td>
<td>Standard A (Negative Control)</td>
<td>2 mL</td>
</tr>
<tr>
<td>CAL B</td>
<td>Standard B (Cut-Off Standard)</td>
<td>2 mL</td>
</tr>
<tr>
<td>CAL C</td>
<td>Standard C (Weak Positive Control)</td>
<td>2 mL</td>
</tr>
<tr>
<td>CAL D</td>
<td>Standard D (Positive Control)</td>
<td>2 mL</td>
</tr>
<tr>
<td>SAMPLEDIL</td>
<td>Sample Diluent Buffer</td>
<td>60 mL</td>
</tr>
<tr>
<td>WASHBUF CONC</td>
<td>Wash Buffer (10×)</td>
<td>60 mL</td>
</tr>
<tr>
<td>TMB SUBS</td>
<td>TMB Substrate</td>
<td>15 mL</td>
</tr>
<tr>
<td>TMB STOP</td>
<td>TMB Stop Solution</td>
<td>15 mL</td>
</tr>
<tr>
<td></td>
<td>Plastic foils</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Plastic bag</td>
<td>1</td>
</tr>
</tbody>
</table>

**Storage and Stability** (refer to the expiry date on the outer box label)
Store kit components at 2-8°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.
5.1. Mikrotiter Strips
12 strips with 8 breakable wells each, coated with Polio antigen (Current vaccine of purified virus material (mixed of types 1 and 3), strain: Sabin). Ready-to-use.

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

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

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

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

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

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

5.8. Stop Solution
15 mL, 0.5 M sulfuric acid. 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 deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

5.11. Plastic Foils
2 pieces to cover the microtiter strips during the incubation.

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

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
- Deionized water
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 7 days. 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 deionized 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.
- 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 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. Rests 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. Rests 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.
9. Evaluation

Example

<table>
<thead>
<tr>
<th></th>
<th>OD Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>0.049</td>
</tr>
<tr>
<td>Cut-Off Standard</td>
<td>0.507</td>
</tr>
<tr>
<td>Weak Positive Control</td>
<td>0.998</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1.756</td>
</tr>
</tbody>
</table>

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 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.

9.2. Quantitative Evaluation

The ready-to-use standards and controls of the Poliovirus IgG ELISA 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 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.

Standard 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

<table>
<thead>
<tr>
<th>Assay Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poliomyelitis ELISA IgG</td>
<td></td>
</tr>
<tr>
<td>Intra-Assay-Precision</td>
<td>7.4 %</td>
</tr>
<tr>
<td>Inter-Assay-Precision</td>
<td>8.8 %</td>
</tr>
<tr>
<td>Inter-Lot-Precision</td>
<td>3.0 – 14.1 %</td>
</tr>
<tr>
<td>Analytical Sensitivity</td>
<td>1.8 U/mL</td>
</tr>
<tr>
<td>Recovery</td>
<td>82 – 86 %</td>
</tr>
<tr>
<td>Linearity</td>
<td>71 – 117 %</td>
</tr>
<tr>
<td>Cross-Reactivity</td>
<td>No cross-reactivity to Bordetella, Diphtheria, Measles, Mumps and Tetanus.</td>
</tr>
<tr>
<td>Interferences</td>
<td>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</td>
</tr>
<tr>
<td>Clinical Specificity</td>
<td>100 %</td>
</tr>
<tr>
<td>Clinical Sensitivity</td>
<td>98.4 %</td>
</tr>
</tbody>
</table>

11. References

2. CDC. Progress towards global poliomyelitis eradication, 1996. MMWR 1997; 46: 579-84
4. Ärzte Zeitung, 04.02.1998
7. Alexander und Raettig: Infektionskrankheiten. Thieme Verlag, Stuttgart
Symbols / Symbole / Symbôles / Símbolos / Σύμβολα

| Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: | Αριθμός-Κατ.: |
| Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote n.: / Αριθμός -Παραγωγής: |
| Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από: |
| Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα |
| Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο |
| In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση. |
| Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l’abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου. |
| Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους: |
| Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός: |
| Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή! |

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or verbal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

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