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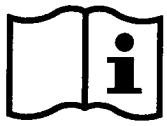

Useŕs Manual

# Haemophilus Influenzae B IgG ELISA

Enzyme Immunoassay for the determination of IgG-antibodies against Polyribosylribitolphosphate (PRP) of Haemophilus influenzae Type B in human rerum, citrate-, EDTA- or heparin plasma

IVD

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## 1. INTRODUCTION

*Haemophilus influenzae* type B (HiB) is a very common cause of invasive critical infectious diseases in children up to the age of six. Following infection the symptoms of the disease include: Pericarditis, osteomyelitis, meningitis, encephalitis, pneumonia, sinusitis and otitis. In many cases the disease is lethal or leads to neurological damage, which cannot always be prevented by rapid antibiotic therapy.
The underlying reason for the disease is very often a latent immunodeficiency with a specifically reduced humoral immune response to the polyribosylribitolphosphate (PRP) in the polysaccharide encapsulation of the bacterium. In children another reason is the immaturity of the immune system. The term "immunocompromised patients" is used, comprising all acquired and innate specific and unspecific immunodeficiencies.
As a result, in children of 3 months of age or older a vaccination with different sorts of PRP-containing vaccines is recommended. This can lead to a clear reduction in the number of infections with *Haemophilus influenzae* type B.
The titer of antibodies produced by vaccination can be used to confirm whether the vaccination has been successful. HiB IgG is used to measure the level of PRP-specific IgG-antibodies following a 4-6 week period after complete immunization to monitor the humoral immune status of children or other individuals at risk.

## 2. INDICATIONS

- Monitoring of the humoral immunostatus after vaccination.
- Verification of the diagnosis *Haemophilus influenzae* type B infection by repeated monitoring of antibody concentrations.
- Risk assessment in immunocompromised patients leading to a failure of vaccination with a PRP-containing vaccine.

This group comprises:

- Children under 2 years having had an infection with *Haemophilus influenzae* type B,
- children with chronic, recurring bacterial infections of the respiratory tract,
- children with chronic otitis,
- patients with confirmed humoral immunodeficiencies (IgG2-deficiency, IgA-deficiency),
- patients with confirmed granulocyte deficiencies, patients under chemo or cytostatic therapy,
- children after splenectomy, patients with sickle-cell anaemia,
- patients with trisomy 21 (Down) syndrome, and certain ethnic groups.

## 3. TEST PRINCIPLE

HiB-IgG is a two-step-ELISA. The wells in the ELISA test strips are coated with PRP. During incubation of diluted serum or plasma samples specific antibodies against PRP bind to the solid phase (**sample incubation**). Following a wash procedure all unbound and non-specific components are washed away. During the second incubation step, the **conjugate reaction**, a peroxidase-conjugated anti-human IgG-antibody (anti- human-IgG-HRP) labels specifically bound IgG. In the following wash procedure unbound conjugate is removed. In a third incubation step the **substrate reaction** takes place. The peroxidase part of the bound conjugate oxidizes tetramethylbenzidine (TMB) to a blue substance. This reaction is stopped by adding acidic solution and the colour changes to yellow. The colour intensity is directly proportional to the concentration of the PRP-specific antibodies. The absorbance is measured with an ELISA reader at 450 nm. The antibody concentration in the sample can be determined using a reference curve or ELISA software.

## 4. MATERIALS AND REAGENTS REQUIRED BUT NOT PROVIDED

- Distilled water
- Tubes for dilution
- Graduated cylinder (1000 ml)
- Precision pipette (20, 100, 500 and 1000 µl)
- Pipettes (10 und 20 ml)
- Multichannel pipette or dispensing pipettes (50 and 200 µl)
- Sample mixer
- Timer
- ELISA reader, 450 nm filter

## 5. MATERIALS AND REAGENTS PROVIDED

1. **MTP**, 12 ELISA test strips, with 8 single break wells each coated with PRP from *Haemophilus influenzae* Typ B; sealed in an aluminum bag with desiccant. Ready to use!
2. **WASH 10x**, Wash solution concentrate (10x), 1 bottle, 100 ml per bottle; 0.1 M Tris/HCl pH 7.4, contains detergent and preservative. Dilute before use!
3. **DIL**, incubation buffer; 0.01 M Tris/HCl; pH 7,4; contains detergent and preservative; colored red; 1 bottle; 100 ml. Ready to use!
4. **CAL 1**, **CAL 2**, **CAL 3**, **CAL 4**, **CAL 5**; Calibrators 1-5 ; human sera with stabilizer and preservative; 1 bottle each, 0.2 ml. **Concentrations are lot specific as indicated on the accompanied lot- specific certificate.** Dilute before use!
5. **POS LL**, **POS HL**, Positive control sera, LL, “Low Level”, HL, “High Level”; for testing accuracy; human sera with stabilizer and preservative; 1 bottle each, 0.2 ml. **Concentrations are lot specific and indicated on the accompanied lot-specific certificate.** Dilute before use!
6. **CON**, Conjugate; anti-human-IgG-peroxidase; colored blue; 1 bottle, 0.3 ml. Dilute before use!
7. **S**, Substrate; tetramethylbenzidine (TMB); 1 x 12 ml. Ready to use!
8. **STOP**, Stop solution; 1 N acidic solution; 1 bottle, 15 ml. Ready to use!
9. Adhesive foils; for covering ELISA test strips; 2 pieces. Lot-specific certificate

## 6. TEST PERFORMANCE

### 6.1 Sample Material and Storage

Human serum, citrated, EDTA or heparin plasma. Samples can be stored at 2-8°C up to 6 weeks [12]. Samples can be stored undiluted for several months at temperatures of at least -20°C. Avoid repeated freezing/thawing.

### 6.2 Preparations

Before starting the test, bring all the required components to room temperature (20-26°C).

Dilution of calibrators 1-5, positive control sera and samples, **1+25:**
Example: Add 20 µl to 500 µl **incubation buffer** For the determination by reference curve calibrators 1-5 and control sera are needed.

Preparation of conjugate working solution, **1+100:**
Example for 8 wells: Add 20 µl conjugate to 2000 µl **incubation buffer**. Prepare immediately before needed! Ready to use solution is stable for 60 min at room temperature (20-26°C).

Preparation of wash buffer, **1+9**:
Example for 12 x 8 well strips: Add 30 ml wash solution concentrate (10x) to 270 ml distilled water. Mix thoroughly! Ready to use solution can be stored at 2–8°C for 2 months.

### 6.3 Stability of Reagents

Store test kit and components at 2-8°C. The unopened reagents are stable until the expiry date indicated.

Stability after opening:
6 months after opening at 2-8°C:
**WASH 10x**, **S**, **DIL**, **MTP**, (place the unused microassay test strips in the aluminum bag)
**CAL 1**, **CAL 2**, **CAL 3**, **CAL 4**, **CAL 5**,
**POS LL**, **POS HL**, **CON**

### 6.4 Assay Procedure

**Sample incubation:** Pipette **100 µl** diluted calibrator/control/sample per well. Calibrators should be placed in strips 1 and 2. Cover strips with adhesive foil. Incubate at **room temperature (20-26°C) for 60 min**.

**Wash:** Empty microassay strips and fill each well with **250 µl** ready to use wash buffer. Empty wells again and repeat this wash step twice. Remove excess liquid by tapping the strips onto absorbent paper.

**Conjugate incubation:** Pipette **100 µl** ready to use conjugate per well. Cover with adhesive foil. Incubate at **room temperature (20-26°C) for 60 min**.

**Wash:** Empty microassay strips and carry out wash steps as described above (**3x250 µl** per well).

**Substrate reaction:** Pipette **100 µl** ready to use substrate per well, incubate at **room temperature (20-26°C) for 30 min**.

**Stop reaction:** Pipette **100 µl** of stop solution per well, shake for 10 sec, measure color within 10 min at 450 nm (reference wavelength at 650 nm).

## 7. NOTES FOR THE USER

For professional use.

**Precision and recovery, depend on the following critical factors:**

- Hemolytic, lipemic, icteric or microbial contaminated samples could cause false results.
- Perform the incubations at room temperature (20-26°C). Maintain an exact pipetting sequence.
- Run test in duplicate.
- Incubation periods should not be exceeded by more than ±10%. Incubation period starts **after** the last pipetting step.
- The time to pipette the samples should not exceed 60 sec for each ELISA test strip.
- The time to pipette conjugate, substrate and stop solution should not exceed 10 sec for each ELISA test strip.

**Security notes:**

Stop solution (acidic solution) and components of substrate may cause skin irritations. If acid or substrate should come into contact with eyes, rinse out immediately with plenty of water and consult a physician! The human plasma used in this product has been tested for Human Immunodeficiency Virus (HIV 1+2), Hepatitis B and C and found to be negative (not repeatedly reactive). However, all human blood products should be considered to be potentially infectious. Observe universal precautions concerning the handling of potentially infectious material. Some of the reagents contain preservatives (e. g. ProClin® 300). Do not swallow! Avoid any contact with skin or mucous membranes! Safety data sheet is available on request!

**Disposal considerations**

Product: Chemicals must be disposed of in compliance with the respective national regulations. Disposal of biological components should be in accordance with existing disposal practices employed for patient serum samples or infectious waste.

Packaging: Packaging must be disposed of in compliance with the country-specific regulations. Handle contaminated packaging in the same way as the product itself. If not officially specified differently, non- contaminated packaging may be treated like household waste or recycled.

**Measures after damage on transport**

If a kit is considerably damaged, please contact the manufacturer or local distributor. Do not use considerable damaged components for a test procedure. Store such components or kits until the complaint is handled.

## 8. CALCULATION OF RESULTS

### 8.1 Plotting of a reference curve

Use a semi-logarithmic evaluation sheet for plotting:

x-axis (log): Concentrations in [µg/ml],

y-axis (lin): Absorbance (optical density).

Calculate mean values of calibrators, control sera and sample measurements each. Plot the mean values of calibrators on the evaluation sheet and connect points with a curved ruler.

If evaluation software is used, a program for multiple and non-linear regression (for instance 4-parametric curve adaptation, eq. 1) is recommended.

$$\text{Eq. 1: } Y = d + (a-d)/(1 + (x/c)^b)$$

### 8.2 Quality control

The absorbance of the highest calibrator should be between 1.0 and 2.5. The absorbance of calibrator 1 should be < 0.5. The difference in the absorbance of calibrators 5 and 1 should be at least 1.0. The concentration ranges of the control sera are indicated on the lot-specific certificate. The obtained values are used to check the validity of the test.

### 8.3 Determination of sample concentration

For samples diluted 1+25 the sample (plasma/serum) concentration can be read directly from the reference curve or calculated by the used ELISA software. Samples with an absorbance exceeding calibrator 5 should be prediluted (1+1) with incubation buffer. The concentrations thus obtained have to be multiplied by the dilution factor 2. Concentration values obtained from citrated plasma must be multiplied by factor 1.1.

## 9. INTERPRETATION OF TEST RESULTS

The determination of PRP-specific antibodies shows the level of humoral immune reaction after an immunization with a PRP-containing vaccine or a clinical apparent or inapparent infection with *Haemophilus influenzae* type B. When this test is negative and the patient belongs to a risk group (conf. "Indications"), it can be assumed that a risk for an infection with *Haemophilus influenzae* type B exists.
Humoral immunoreactions after an infection with non- typeable *Haemophilus influenzae* are not detected by HiB IgG.
The failure to seroconvert after vaccination is indicative of the ability to react to bacterial carbohydrate antigens. A polysaccharide-specific immunodeficiency can be observed in patients with chronic bronchitis, recurring pneumoniae, intrinsic bronchial asthma or bronchiectases of unclear genesis.
It has been shown in publications [2, 8, 9, 10] that an antibody concentration under 0.15 µg/ml gives insufficient protection against *Haemophilus influenzae* type B. Antibody concentrations between 0.15 and 1.0 µg/ml indicate that the patient has been immunised with PRP or had an infection with HiB. But only antibody concentrations over 1.0 µg/ml represent a sufficient natural immunity or an aquired protection after the third vaccination.

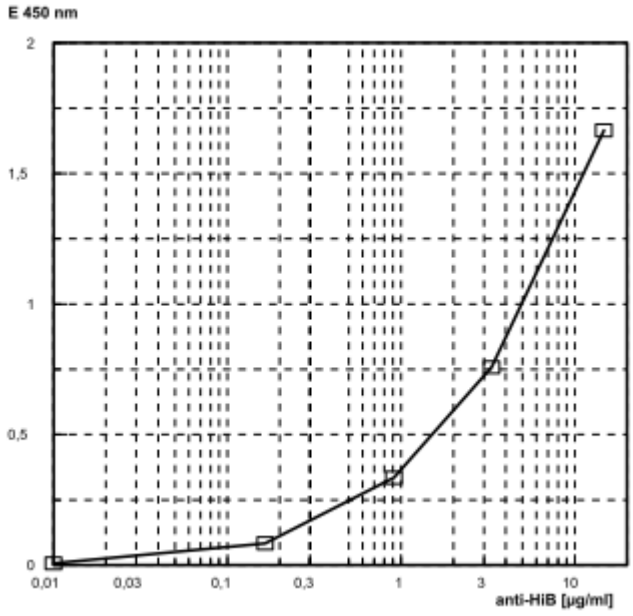
## 10. TEST CHARACTERISTICS

**Intraassay variation**:
2 samples in the concentration range of the calibrators measured 20 times each with one lot in double determination. The intraassay variation was ≤10%.

**Interassay variation**:
**POS HL** and **POS LL** controls measured in 20 runs on different days with one lot in double determination. The interassay variation was between 9 and 12 % respectively.

**Detection limit**: ≤ 0,1 µg/ml.

**Reference Curve (Example):**



## 11. LITERATURE AND REFERENCES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## SYMBOLS USED WITH DEMEDITEC 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-Konfirmitätskennzeichnung | 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 | Mindesthaltbarkeitsdatum | 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 |