# Corynebacterium diphtheriae

IgG – ELISA

Enzyme immunoassay for the quantitativ determination of IgG-class antibodies against Corynebacterium diphtheriae toxin in human serum
Only for in-vitro diagnostic use

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Product Number: CORG0090 (96 determinations)

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## 1. INTRODUCTION

Corynebacteria are aerobic non spore-forming gram-positive rods of irregular shape (0.5 –1 µm thick and 2-6 µm long). They comprise skin commensals, opportunist pathogens and several major pathogens, including Corynebacterium diphtheriae. In general, they are isolated from throat swabs on selective media containing tellurite. The bacterial infection caused by C. diphtheriae, Diphtheria, has two forms. Respiratory diphtheria is typically caused by toxin-producing (toxigenic) strains; cutaneous disease can be caused by either toxigenic or nontoxigenic strains. In the respiratory form of the disease, a membrane is formed; this membrane is usually visible on the throat or tonsils. Persons may die from asphyxiation when the membrane obstructs breathing. Other complications are caused by remote effects of the diphtheria toxin (myocarditis, nerve paralysis) Cutaneous diphtheria is usually mild, typically consisting of non-distinctive sores or shallow ulcers and only rarely involving toxic complications (1-2% of infections with toxigenic strains). Diphtheria was one of the most common causes of death among children during the prevaccine era.
Since the introduction and widespread use of diphtheria toxoid vaccine (formalin-inactivated diphtheria toxin) in most industrialized countries the disease is now characterized by sporadic cases and intermittent outbreaks of low intensity. But recent large epidemics of diphtheria in several eastern European countries have again drawn attention to this „forgotten“ disease – and, the majority of these cases have occurred among adolescents and adults instead of children.

| Species | Disease | Symptoms | Mechanism of Infection |
|---|---|---|---|
| Corynebacterium diphtheriae | Diphtheria (respiratory) | sore throat and low-grade fever<br>swelling of the neck (“bull neck”) from inflammation<br><br>Complications: exotoxin-induced damage to other organs | Transmission from person to person through close physical and respiratory contact<br><br>Transmission is increased in overcrowded and poor socio-economic conditions |

The only effective way to control diphtheria is by prophylactic immunization with diphtheria toxoid. Antibody to the toxoid protects against the action of the toxin; immunized persons can be infected by toxin-producing strains of diphtheria, but the systemic manifestations of diphtheria do not occur. The outcome of the disease improves with early, appropriate treatment. Prompt recognition and reporting of the disease is important to assure early, appropriate treatment with diphtheria anti-toxin. Infection may be identified by

- Microscopy: Gram stain
- Serology: Detection of toxin production by ELISA

## 2. INTENDED USE

The NovaTec Corynebacterium diphtheriae IgG-ELISA is intended for the quantitative determination of IgG class antibodies against Corynebacterium diphtheriae toxin in human serum. This allows the determination of the immune status of the patients facilitating individual recommendations about the necessity of a basic immunization or booster injection.

## 3. PRINCIPLE OF THE ASSAY

The quantitative immunoenzymatic determination of IgG-class antibodies against C. diphtheriae toxin is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterstrip wells are precoated with inactivated specific Corynebacterium diphtheriae toxin (toxoid) antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human IgG conjugate is added. This conjugate binds to the captured C.diphtheriae toxin-specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of C. diphtheriae toxin-specific IgG antibodies in the specimen. Sulfuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance at 450 nm is read using an ELISA microwell plate reader.

## 4. MATERIALS

### 4.1. Reagents supplied

- **C. diphtheriae toxin Coated Wells (IgG):** 12 breakapart 8-well snap-off strips coated with C. diphtheriae toxin (toxoid) antigens; vacuum sealed, in resealable aluminium foil.
- **IgG Sample Diluent*****: 1 bottle containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2; colored yellow; ready to use; white cap.
- **Stop Solution**: 1 bottle containing 15 ml sulfuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Solution (20x Concentrate)*:** 1 bottle containing 50 ml of a 20-fold concentrated buffer for washing the wells; pH 7.2 ± 0.2; white cap.
- **C. diphtheriae toxin anti-IgG Conjugate**:** 1 bottle containing 20 ml of peroxidase labelled antibodies to human IgG; colored red; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.
- **C. diphtheriae toxin IgG Standards***:** 4 vials, each containing 2ml, colored yellow; ready to use:

| | | |
|---|---|---|
| Standard A: | 0.000 | IU/ml; blue cap |
| Standard B: | 0.015 | IU/ml; green cap |
| Standard C: | 0.075 | IU/ml; yellow cap |
| Standard D: | 0.150 | IU/ml; red cap |

* contains 0.01 % Thimerosal after dilution
** contains 0.2 % Bronidox L
*** contains 0.1 % Kathon

### 4.2. Materials supplied

- 1 Strip holder
- 2 Cover foils
- 1 Test protocol
- 1 distribution and identification plan

### 4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

## 5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2…8 °C.

## 6. REAGENT PREPARATION

*It is very important to bring all reagents, samples and standards to room temperature(20…25°C) before starting the test run!*

### 6.1. Coated Snap-off Strips

The ready to use breakapart snap-off strips are coated with anti-human IgG-class antibodies. Store at 2...8°C. The strips are vacuum sealed. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the dessiccant supplied and stored at 2… 8 °C; stability until expiry date.*

### 6.2. C. diphtheriae toxin anti-IgG Conjugate

The bottle contains 20ml conjugate with the components anti-human IgG horseradish peroxidase, buffer, stabilizers, preservatives and an inert red dye. The solution is ready to use. Store at 2...8°C. *After first opening stability until expiry date when stored at 2…8°C.*

### 6.3. Standards

The vials labelled with Standard A, B, C and D contain a ready to use standard solution. The concentration of the standards, calibrated in accordance with the 3rd International Standard of the WHO, are:

| | | |
|---|---|---|
| Standard A: | 0.000 | IU/ml |
| Standard B: | 0.015 | IU/ml |
| Standard C: | 0.075 | IU/ml |
| Standard D: | 0.150 | IU/ml |

The solutions have to be stored at 2...8°C and contain 0.1% Kathon. *After first opening stability until expiry date when stored at 2…8°C.*

### 6.4. IgG Sample Diluent

The bottle contains 100ml phosphatebuffer, stabilizers, preservatives and an inert yellow dye. It is used for the dilution of the patient specimen. This ready to use solution has to be stored at 2...8°C. *After first opening stability until expiry date when stored at 2…8°C.*

### 6.5. Washing Solution (20xconc.)

The bottle contains 50ml of a concentrated buffer, detergents, stabilizers and preservatives. Dilute washing solution 1+19; e.g. 10 ml washing solution + 190 ml fresh and germ free redistilled water. The diluted buffer will keep for at least four weeks if stored at 2...8°C. *Crystals in the solution disappear by warming up to 37 °C in a water bath.*

### 6.6. TMB Substrate Solution

The bottle contains 15ml of a Tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C, away from the light. *The solution should be colourless or have a slight blue tinge. If the substrate turns intor blue, it may have become contaminated and should be discharged*. *After first opening stability until expiry date when stored at 2…8°C.*

### 6.7. Stop Solution

The bottle contains 15ml 0.2 M sulphuric acid solution (R 36/38, S 26), ready to use ,store at 2...8°C. After first opening stability until expiry date..

## 7. SPECIMEN COLLECTION AND PREPARATION

Use human serum samples with this assay. If the assay is performed within 24 hours after sample collection, the specimen should be kept at 2...8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. *Avoid repeated freezing and thawing.*

### 7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10µl sample and 1ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

For patients with expected **antitoxin concentrations greater than Standard D** (0.15 IU/mL) a second 1 + 10 dilution of this 1 + 100 diluted patient sample should be performed; e.g. 20 µL of first sample dilution + 200 µL of IgG sample dluent (mix well). Dilution factor: 11

*Standards are ready to use and must not be diluted.*

## 8. ASSAY PROCEDURE

### 8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and standards should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

| | | |
|---|---|---|
| 1 well | (e.g. A1) | for the substrate blank, |
| 4 wells | (e.g. B1, C1, etc.) | for Standard A, B, C and D. |

*It is recommended to detemine patient samples in duplicate.*

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard and sample.

Adjust the incubator to 37° ± 1°C.

1. Dispense 100µl of each Standard (A, B, C and D) and diluted sample into the respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37±1°C.**
4. When incubation has been completed, remove the foil, aspirate the content off the wells and wash each well five times with 300µl of washing solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   *Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.*
5. Dispense 100µl C. diphtheriae toxin anti-IgG Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature (20…25°C).** *Do not expose to direct sunlight.*
7. Repeat step 4.
8. Dispense 100µl TMB Substrate Solution into all wells
9. **Incubate for exactly 15 min at room temperature (20…25°C) in the dark.**
10. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. *Any blue color developed during the incubation turns into yellow.*

    *Note: Highly positive patient samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Dilute the specimen as mentioned under 7.1. Sample Dilution.* Measure the absorbance of the specimen at 450/620nm within 30 min after addition of the Stop Solution.

### 8.2. Measurement

Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.

*If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!*

Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard and patient sample in the distribution and identification plan.

*Dual wavelength reading using 620 nm as reference wavelength is recommended.*

Where applicable calculate the mean absorbance values of all duplicates.

## 9. RESULTS

### 9.1. Assay Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank** in A1: Absorbance value **lower than 0.100.**
- **Standard A** in B1: Absorbance value **lower than 0.300.**

## 9.2. Calculation of Results

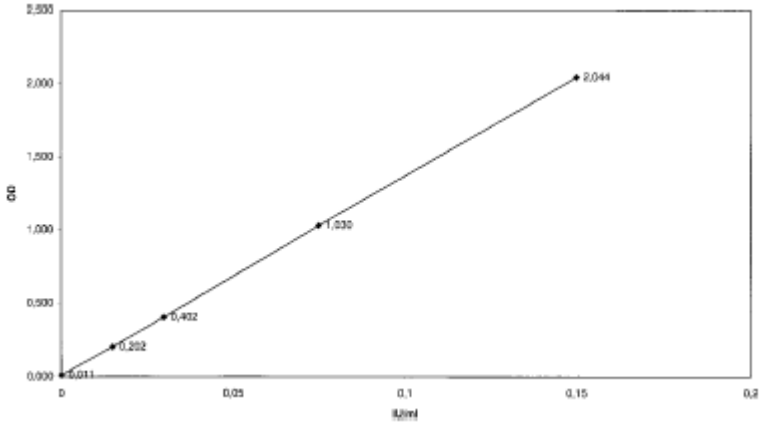
In order to obtain **quantitative results in IU/ml** plot the (mean) absorbance values of the 4 Standards A, B, C and D on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0.000, 0.015, 0.075, 0.150 IU/ml) and draw a standard calibration curve (absorbance values on the vertical y-axis, concentrations on the horizontal x-axis).

Read results from this standard curve employing the (mean) absorbance values of each patient specimen and control.

*Note: Readings of additionally (1+10) diluted patient samples must be multiplied by the appropriate dilution factor in order to obtain correct results! (Dilution: 1+10 = Dilution factor: 11).*

All suitable computer programs available can be used for automated result reading and calculation.

## 9.3. Typical Calibration Curve




## 9.4. Interpretation of Results and Recommendations [ IU/ml]

Each result should be carefully assessed by a physician.

| | |
|---|---|
| < 0.01 | **No protective antibody level!**<br>Immediate full course of basic immunization is recommended! |
| 0.01 - 0.09 | **No reliable protection!**<br>Immediate booster injection and control of antibody concentration 4 to 6 weeks later is recommended. |
| 0.1 – 1.0 | **Immunity in most cases!**<br>Possibly booster injection and control of antibody concentration 4 to 6 weeks later. |
| > 1.0 - 1.5 | **Reliable protection!**<br>After about 5 years control and booster injection is recommended. |
| > 1.5 - 2.0 | Reliable **long term protection:**<br>After about 7 years control and booster injection is recommended. |
| > 2.0 | Range of **long term** protection:<br>After about 10 years control and booster injection is recommended. |
| | It is recommended that the basic immunisation or booster is checked 4-6 weeks after immunisation and to record the data on the certificate of vaccination. |

## 10. SPECIFIC PERFORMANCE CHARACTERISTICS

### 10.1. Precision

| Intraassay | n | Mean value | CV (%) |
|---|---|---|---|
| Standard B | 5 | 0.33 | 12 |
| Standard C | 5 | 1.51 | 4.7 |
| Standard D | 6 | 2.56 | 1.6 |

| Interassay | n | Mean value | CV (%) |
|---|---|---|---|
| Standard B | 14 | 0.34 | 10.1 |
| Standard C | 15 | 1.54 | 5.5 |
| Standard D | 16 | 2.58 | 4.2 |

### 10.3. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 84.6%

### 10.4. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100%.

### 10.5 Analytical sensitivity

The analytical sensitivity – defined as the apparent concentration of the analyte that can be distinguished from the zero calibrator –is 0.01 IU/ml.

## 11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromized patients and newborns serological data only have restricted value.

## 12. PRECAUTIONS AND WARNINGS

- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

| | |
|---|---|
| WARNING: | Thimerosal is toxic! Do not swallow. Avoid contact with skin and mucous membranes! |
| WARNING: | In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes! |
| WARNING: | Sulfuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor! |

## 13. LITERATURE

BGA,Impfempfehlung der Ständigen Impfkommission (STIKO)des Bundesgesundheitsamtes vom 22.2.1994, Bundesgesundheitsblatt 8/94

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Hofmann,F.,F.Schuh,M.Michaelis,U.Stößel (1994):Zur Akzetanz von Schutzimpfungen bei Ärzten und bei der Allgemeinbevölkerung,Ges.Wes.56,371-376

WHO (7.5.1993):Expanded Programme on Immunization-Outbreak of diphtheria,update Wkly Epid Rec No.19,134-138

WHO (26.8.1994):Expanded Programme on Immunization-Diphteria Epidemic.Wkly Epid Rec Nr.34

## 14. ORDERING INFORMATION

Prod. No.: CORG0090 C. diphtheriae toxin IgG-ELISA (96 Determinations)

# SCHEME OF THE ASSAY

C. diphtheriae toxin IgG-ELISA

## Assay Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and standards on the result sheet supplied in the kit
Select the required number of microtiter strips or wells and insert them into the holder.

## Assay Procedure

| | Substrate blank (z.B. A1) | Standard A | Standard B | Standard C | Standard D | Sample (1+100 diluted) |
|---|---|---|---|---|---|---|
| Standard A | - | 100µl | - | - | - | - |
| Standard B | - | - | 100µl | - | - | - |
| Standard C | - | - | - | 100µl | - | - |
| Standard D | - | - | - | - | 100µl | - |
| Sample (1+100 diluted) | - | - | - | - | - | 100µl |
| Cover wells with foil supplied in the kit<br>**Incubate for 1 h at 37°C**<br>Wash each well three times with 300µl of washing solution | | | | | | |
| Conjugate | - | 100µl | 100µl | 100µl | 100µl | 100µl |
| Cover wells with foil supplied in the kit<br>**Incubate for 30 min at room temperature**<br>Wash each well three times with 300µl of washing solution | | | | | | |
| TMB Substrate | 100µl | 100µl | 100µl | 100µl | 100µl | 100µl |
| **Incubate for15 min at room temperature in the dark** | | | | | | |
| Stop Solution | 100µl | 100µl | 100µl | 100µl | 100µl | 100µl |
| Photometric measurement at 450 nm (reference wavelength: 620 nm) | | | | | | |


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