NovaTec Immundiagnostica GmbH

Declaration of Conformity

We NovaTec Immundiagnostica GmbH
Waldstraße 23 A6
63128 Dietzenbach
Germany

herewith declare under our own responsibility, that the product

NovaLisa® Corynebacterium diphtheriae toxin IgG (CORG0090)

and the following components:

MTP	Microtiterplate
DIL G	IgG Sample Dilution Buffer
SOLN STOP	Stop Solution
WASH BUF 20x	Washing Buffer (20x conc.)
CONJ	Conjugate
SUB TMB	TMB Substrate Solution
CAL A - D	Standards

is in accordance with the requirements of the IVD Directive 98/79/EC of the European Parliament and Council of Oct. 27, 1998 in regard to in vitro diagnostic medical devices (IVDs).

The accordance was shown by conformity assessment procedures in
Annex III (2-5)

Dietzenbach 2020.07.22

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Jennifer Vaiger
Quality Management Representative

The conformity of the above mentioned product is checked at least every 3 years. This is documented by rechecking and signing the general requirements.

NovaTec Immundiagnostica GmbH

NovaLisa®

Corynebacterium diphtheriae toxin IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: CORG0090 (96 Determinations)

ENGLISH

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.

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 of the disease is important to assure early, appropriate treatment with diphtheria anti-toxin

Species	**Disease**	**Symptoms (e.g.)**	**Transmission route**
Corynebacterium diphtheriae	Diphtheria (respiratory)	with malaise, sore throat, anorexia, low-grade fever and swelling of the neck (“bull neck”) from inflammation. Complications: exotoxin-induced damage to other organs.	Transmission from person to person through close physical and respiratory contact Transmission is increased in overcrowded and poor socio-economic conditions

Infection or presence of pathogen may be identified by:

- Microscopy
- Serology: e.g. by ELISA

2. INTENDED USE

The Corynebacterium diphtheriae toxin IgG ELISA is intended for the quantitative determination of IgG class antibodies against Corynebacterium diphtheriae toxin in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The quantitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. 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 specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break apart 8-well snap-off strips coated with Corynebacterium diphtheriae toxin (toxoid) antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0,1 %; ready to use; yellow cap.
- **Standards:** 4 vials, each containing 2 mL standard; coloured yellow; ready to use; ≤ 0.02% (v/v) MIT.

  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

  The standards are calibrated in accordance with the ‘‘1st International Standard for Diphtheria Antitoxin Human IgG (WHO, 2012).

For hazard and precautionary statements see 12.1

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened 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 and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Corynebacterium diphtheriae toxin (toxoid) antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

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 could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

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

8. ASSAY PROCEDURE

Please read the instruction for use carefully before performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

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

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

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the 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 of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature(20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value 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/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate blank: Absorbance value < 0.100**
- **Standard A: Absorbance value < 0.200**
- **Standard B: Absorbance value > 0.100**
- **Standard C: Absorbance value > 0.500**
- **Standard D: Absorbance value > 1.000**

Standard A < Standard B < Standard C < Standard D

If these criteria are not met, the test is not valid and must be repeated.

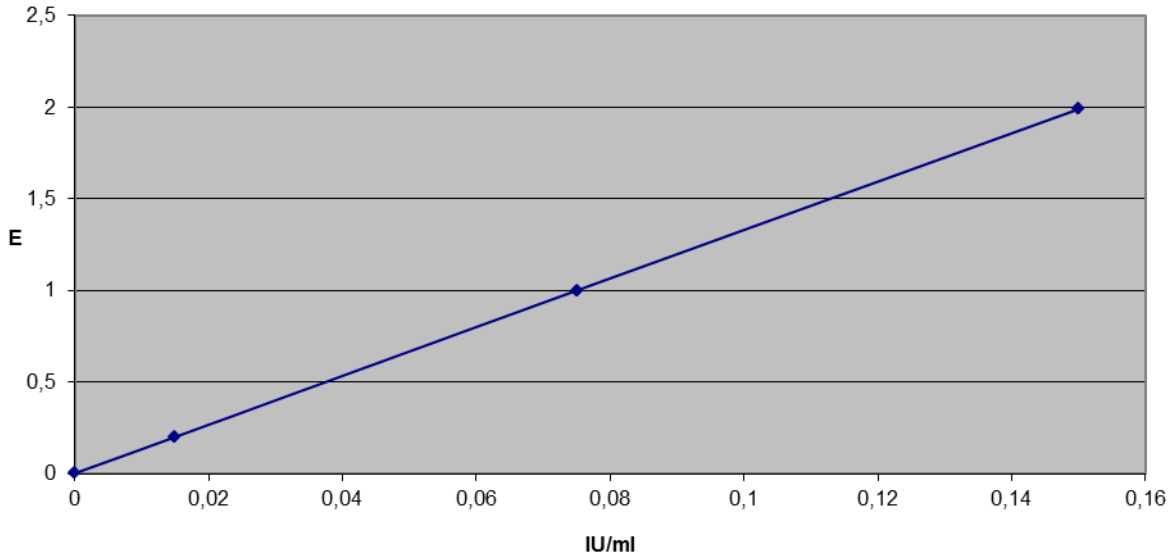
9.2. Calculation of Results

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 y-axis, concentrations on the x-axis).

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

For the calculation of the standard-curve mathematical Point to Point function should be used.

9.3. Typical standard Curve



9.4. Interpretation of Results

according to: RKI 1999

< 0.01 IU/mL	No protective antibody level! Immediate full course of basic immunization is recommended!
0.01 - 0.09 IU/mL	No reliable protection! Immediate booster injection is recommended.
0.1 – 1.0 IU/mL	Reliable protection!
> 1.0 IU/mL	Reliable long term protection: After about 10 years after last booster 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.

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 immunocompromised patients and newborns serological data only have restricted value.

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	n	Mean value (E)	CV (%)
#1	24	1,347	3,85
#2	24	1,843	3,86
#3	24	0,527	3,02

Interassay	n	Mean value (IU/mL)	CV (%)
#1	12	7,83	12,95
#2	12	34,47	6,99
#3	12	35,39	6,86

10.2. 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 100% (95% confidence interval: 89.42% - 100%).

10.3. 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% (95% confidence interval: 95.44% - 100%).

10.4. Analytical Sensitivity

The analytical sensitivity (according to CLSI EP17-A) is defined as the apparent concentration of the analyte that can be distinguished from the zero calibrator. It is 0.00092 IU/mL.

10.5. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.6. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

10.7. Measurement range

The measurement range is 0.00092 IU/mL – 0.15 IU/mL.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- 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.
- Do not interchange reagents or Microtiterplates 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/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

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