Serazym® Clostridium difficile Toxin A+B

Enzyme immunoassay for detection of *Clostridium difficile* Toxin A and B
in stool specimens and culture suspensions

REF E-040 96 REF E-040-A2 2x 96 IVD *In-vitro*- diagnostic medical device CE

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Introduction

Clostridium difficile is a bacterium causing nosocomial diarrhea in adults during or after the treatment with antibiotics such as 3rd generation cephalosporines (1). Although 2 - 3% of healthy adults and 20 - 50% of healthy children are colonized with *Clostridium difficile*, the infection is usually of exogenous origin and results from the contact either to hospital staff or to *Clostridium difficile* spores which may contaminate toilets, bed clothes etc. Both exotoxins A and B of this spore-forming bacteria cause the depolymerisation of actin filaments due to the intracellular enzymatic modification of rho-proteins. Consequently, the permeability of cell membrane is raised and neutrophils may invade leading to expression of the clinical picture of the so-called Clostridium difficile-associated diarrhea and colitis or finally the pseudomembraneous colitis (PMC) (1). As the production of toxins and the outbreak of disease is correlated, diagnosis of *Clostridium difficile* infection is based mainly on a direct detection of the toxins in stool specimens. To date the cytotoxicity test has been considered as the gold standard for detection of *Clostridium difficile* toxins. Recently it has been replaced to a large extent by immunological tests such as enzyme immunoassay (2).

References:

1. Rambaud J-C., LaMont J-T. (Hrsg.): “Ökosystem Darm Special - Updates on Clostridium difficile” Springer Verlag 1995
2. Wilkins T.D. and Lyerly D.M. (2003): „Clostridium difficile Testing: after 20 Years, Still Challenging“ Journal Of Clinical Microbiology, Vol. 41, No. 2, p. 531-534

Intended Use

The *Serazym*® Clostridium difficile Toxin A+B is an *in-vitro*-diagnostic medical device for direct detection of the toxins A and B of *Clostridium difficile* in stool specimens and culture suspensions.

Principle of The Test

Serazym® Clostridium difficile Toxin A + B is an indirect two-site-immunoassay for the qualitative determination of both *Clostridium difficile* toxins A and B based on polyclonal and monoclonal antibodies. *Clostridium difficile* toxins of stool specimens or culture suspensions and the positive control react with monoclonal anti-toxin A and polyclonal anti-toxin B antibodies coated on the solid phase of the microplate. After incubation non-bound material is removed by a washing step. Subsequently bound toxins specifically react with biotinylated polyclonal anti-toxin A and monoclonal anti-toxin B antibodies during a second incubation period. Non-bound material is separated from the solid-phase immune complexes by a subsequent washing step. During the next incubation period horseradish peroxidase (HRP) conjugated streptavidin reacts with the bound biotinylated antibodies. Unbound conjugate is removed by a washing step. HRP converts the subsequently added colourless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells turning the solution from blue to yellow. The optical density (OD) of the solution read at 450 / ≥ 620 nm is directly proportional to the specifically bound amount of *Clostridium difficile* toxin A and B. After consideration of the cut-off value, results are interpreted as positive or negative.

Test Components

			For 96 Wells	For 2x 96 Wells
1	WELLS	**Microtitration plate** coated with monoclonal anti-Toxin A- (mouse) and polyclonal anti-Toxin B-antibodies (rabbit)	12 single breakable 8-well strips colour coding red vacuum-sealed with desiccant	2x 12 single breakable 8-well strips colour coding red vacuum-sealed with desiccant
2	WASHBUF CONC 10x	**Wash buffer** 10-fold	100 ml concentrate for 1000 ml solution white cap	2x 100 ml concentrate for 2x 1000 ml solution white cap
3	DIL	**Sample diluent**	100 ml · ready to use coloured yellow black cap	2x 100 ml · ready to use coloured yellow black cap
4	CONTROL +	**Positive control** *C. difficile* Toxin reactive sample	2.0 ml · ready to use coloured blue red cap	4.0 ml · ready to use coloured blue red cap
5	CONTROL –	**Negative control** *C. difficile* Toxin negative sample	2.0 ml · ready to use coloured blue green cap	4.0 ml · ready to use coloured blue green cap
6/1	CONJ BIOTIN	**Biotin-conjugate** Biotinylated, polyclonal anti-Toxin A- (rabbit) and monoclonal anti-Toxin B-antibodies (mouse)	15 ml · ready to use coloured green white cap	30 ml · ready to use coloured green white cap
6/2	CONJ STREPT	**Streptavidin-HRP-conjugate**	15 ml · ready to use coloured red brown cap	30 ml · ready to use coloured red brown cap
7	SUBSTR TMB	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml · ready to use blue cap	28 ml · ready to use blue cap
8	STOP	**Stop solution** 0.25 M sulphuric acid	15 ml · ready to use yellow cap	28 ml · ready to use yellow cap

Preparation And Storage Of Samples

Toxin detection from stool specimens
Collection and storage
Stool samples should be stored at 2…8°C immediately after collection and processed within 72 hours or frozen at at least –20°C. Storage at -20°C as well as repeated freezing and thawing of samples should be avoided.
Formalin-preserved stool samples should not be used in this assay. Stool samples already diluted with the *Serazym*® sample diluent can be stored for up to 72 h at 2…8°C before testing in the ELISA.
Preparation
Warm samples to room temperature and mix thouroughly. Pipette 1000 µl of sample diluent into a clean tube. Using a disposable stirring rod tansfer about 200 mg (diameter about 4 - 6 mm) of faeces if solid or pipette 200 µl if liquid into the tube and suspend thoroughly. If necessary spin down floating particles in a micro centrifuge at maximum speed for 1 min.

Toxin detection from culture suspensions (toxigenic culture)
Colonies of *Clostridium difficile* grown on blood or CCFA agar for 48 hours can be tested directly in the *Serazym*® Clostridium difficile Toxin A+B. Prepare a bacterial suspension according to Mc Farland standard 1 (OD value at 600 nm: 0.20 - 0.25 after zero compensation to the yellow coloured *Serazym*® sample diluent):
Pipette 1000 µl of sample diluent into a clean tube.
Transfer 2 - 4 inoculating loops of a *C. difficile* culture into the sample diluent and suspend on a vortex mixer. Read OD value at 600 nm as described above where required.
Use 100 µl for ELISA testing. If selective culture media are used the detectable amount of toxins may be reduced due to inhibitory components of such media resulting in decreased OD values in the ELISA. Therefore using selective media for toxigenic culture requires the preparation of a bacterial suspension at least according to Mc Farland standard 4 (OD 600 nm > 1.0 after zero compensation to the yellow coloured *Serazym*® sample diluent). In this case the *Clostridium difficile* colonies of at least half of a densely grown agar plate have to be harvested. Where required the recommendations and instructions of the medium manufacturers are to be observed.

Materials Required But Not Provided

Micropipettes · multi-channel pipette or multi-pipette · Reagent container for multi-channel pipette · 8-channel wash comb with vacuum pump and waste bottle or microplate washer or 8-channel pipette · microplate reader with optical filters for 450 nm for measurement and ≥ 620 nm for reference · distilled or deionized water · glassware · tubes (2 ml) for sample preparation · orbital shaker for performance of test variant 2

Preparation And Storage Of Reagents

Kit size and expiry
One kit is designed for 1x 96 or 2x 96 determinations. The expiry date of each component is reported on its respective label, that of the complete kit on the outer box label. Upon receipt, all test components have to be kept at 2…8°C, preferably in the original kit box. After opening all kit components are stable for at least 2 months, provided proper storage. The ready to use wash solution can be used for at least one month when stored at 2…8°C.

Reagent preparation
Allow all components to reach room temperature prior to use in the assay. The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.
Prepare a sufficient amount of wash solution by diluting the 10-fold concentrated wash buffer 1 + 9 with distilled or deionized water.
For Example: 10 ml wash buffer concentrate *(2)* + 90 ml distilled or deionized water.

Assay Procedure

The *Serazym*® Clostridium difficile Toxin A+B can be performed in two ways:

1. Incubation without shaking; complete test duration 2 hours and 15 minutes
2. Incubation with shaking; complete test duration 1 hour and 15 minutes

Dilute samples with sample diluent *(3)* 1 : 6, e.g. 200 mg or 200 µl stool + 1.0 ml sample diluent *(3)* or transfer 2 - 4 inoculation loops of a *C. difficile* colony into a tube with 1.0 ml sample diluent *(3)* and mix thoroughly on a vortex.
Avoid any time shift during dispensing of reagents and samples.
Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle!
Avoid direct light exposure of the TMB substrate solution!

Working steps variant 1: without shaking

1. Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.
2. Pipette: 100 µl **CONTROL +** positive control *(4)*
   100 µl **CONTROL –** negative control *(5)*
   100 µl **diluted stool specimen** or **culture suspension**.
3. Cover plate and incubate for 60 min at RT.
4. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
5. Dispense 3 drops (or 120 µl) **CONJ BIOTIN** biotin-conjugate *(6/1)* per well.
6. Cover plate and incubate for 30 min at RT.
7. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
8. Dispense 3 drops (or 120 µl) **CONJ STREPT** streptavidin-HRP-conjugate *(6/2)* per well.
9. Cover plate and incubate for 30 min at RT
10. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
11. Dispense 3 drops (or 120 µl) **SUBSTR TMB** substrate *(7)* per well.
12. Incubate for 15 min at RT protected from light.
13. Dispense 3 drops (or 120 µl) **STOP** stop solution *(8)* per well, mix gently.
14. Read OD at 450 nm / ≥ 620 nm with a microplate reader within 30 min after reaction stop.

Working steps variant 2: with shaking

1. Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.
2. Pipette: 100 µl **CONTROL +** positive control *(4)*
   100 µl **CONTROL –** negative control *(5)*
   100 µl **diluted stool specimen** or **culture suspension**.
3. Cover plate and incubate for 30 min at RT on an orbital shaker with a frequency of 500-700/min.
4. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
5. Dispense 3 drops (or 120 µl) **CONJ BIOTIN** biotin-conjugate *(6/1)* per well.
6. Cover plate and incubate for 15 min at RT on an orbital shaker with a frequency of 500-700/min.
7. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
8. Dispense 3 drops (or 120 µl) **CONJ STREPT** streptavidin-HRP-conjugate *(6/2)* per well.
9. Cover plate and incubate for 15 min at RT on an orbital shaker with a frequency of 500-700/min.
10. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
11. Dispense 3 drops (or 120 µl) **SUBSTR TMB** substrate *(7)* per well.
12. Incubate for 15 min at RT without shaking protected from light.
13. Dispense 3 drops (or 120 µl) **STOP** stop solution *(8)* per well, mix gently.
14. Read OD at 450 nm / ≥ 620 nm with a microplate reader within 30 min after reaction stop.

Result Interpretation

Qualitative evaluation
Cut-off determination: OD negative control + 0.20
Samples with absorbances higher than the cut-off value are considered positive, samples with absorbances 10% below the cut-off value are considered negative for *Clostridium difficile* toxin A and B antigen. Samples within 10 % below the cut-off up to the cut-off value have to be considered borderline and should be repeatedly tested. In case of repeated borderline result a second sample of the corresponding patient should be investigated.

Reference Values

***Serazym*® C. difficile Toxin A+B**	
Positive	> Cut-off
Borderline	0,9 x Cut-off – Cut-off
Negative	< 0,9 x Cut-off

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters too. Therefore, the mentioned reference values provide a guide only to values which might be expected.

Test validity

The test run is valid if:

- the mean OD of the negative control is ≤ 0.20 (manual performance)
  ≤ 0.30 (automatic performance)
- the mean OD of the positive control is ≥ 1.00

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

Limitations of the procedure

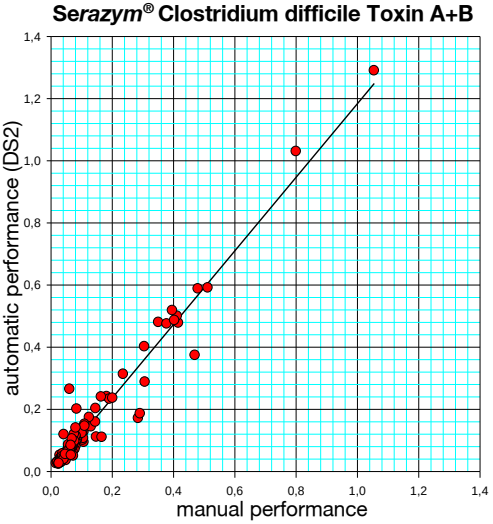
There is no correlation between measured absorbance and seriousness of the infection. It is also not allowed to correlate absorbances of the samples with that of the positive control. Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions, not sufficiently homogenized samples or solid particles after centrifugation of the suspension can cause false negative as well as false positive results. Formalin treated samples may cause false positive results. A negative test result in the *Serazym*® Clostridium difficile Toxin A+B does not exclude an infection: The overall interpretation of the ELISA results should always consider the microbiological examination as well as clinical findings.

Automatic Processing

Performing the *Serazym*® Clostridium difficile Toxin A+B on fully automated microplate processors (e.g. DS2, DSX) may cause elevated absorbances in comparison to the manual procedure due to individual differences concerning wash procedures and general technical specifications of the equipment. In these cases a maximum value of 0.3 absorbance units is permissible for the negative control. It is recommended to use a wash procedure including 10 seconds soak time per strip and wash step followed by a wash step with distilled or deionized water with 10 seconds of soak time after the final wash step of each wash cycle. If necessary the number of washing steps can be enhanced from 5x to 7x-8x.

Correlation: manual – automatic processing

A panel of 125 stool specimens was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with r = 0.976.



Performance Characteristics

Precision

Intra-assay coefficient of variation (CV) in the *Serazym*® Clostridium difficile Toxin A+B calculated from 8-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	1.386	0.042	3.0
2	0.506	0.017	3.3
3	0.332	0.028	8.5

Inter-assay coefficient of variation (CV) in the *Serazym*® Clostridium difficile Toxin A+B in 5 different test runs on 2 different days from 8-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	1.321	0.102	7.7
2	0.485	0.034	6.9
3	0.345	0.037	10.8

Specificity and sensitivity

A total of 154 stool specimens were tested in parallel with the *Serazym*® Clostridium difficile Toxin A+B and another commercially available ELISA.

	comparative ELISA positive	comparative ELISA negative
Serazym® ELISA positive	103	4
Serazym® ELISA negative	2	45

Specificity: 91.8% · Sensitivity: 98.0%

Cross reactivity

Faecal samples positive for one of the following intestinal bacteria did not show any cross reaction in the *Serazym*® Clostridium difficile Toxin A+B:
Staphylococcus aureus, enterotoxin negative*; Staphylococcus aureus,* enterotoxin positive*;* EHEC*; Pseudomonas aeruginosa; Salmonella typhimurium; Salmonella enteritidis; Salmonella spec. Aeromonas hydrophila; Aeromonas caviae; Campylobacter spec.; Hafnia alvei; Yersinia enterocolitica* O:3*.*

Negative stool specimens have been spiked with ≥ 10^8 colony forming units of the following microorganisms and tested negative with the *Serazym*® ELISA (OD 450 / 620 nm < Cut-Off):

Aeromonas hydrophila	(ATCC 7966)
Bacillus cereus	(ATCC 11778)
Bacillus subtilis	(ATCC 6633)
Bacteroides fragilis	(ATCC 25285)
Candida albicans	(ATCC 10231)
Campylobacter coli	(ATCC 33559)
Campylobacter jejuni	(ATCC 33291)
Citrobacter freundii	(ATCC 8090)
Clostridium sordellii	(ATCC 9714)
Enterobacter aerogenes	(ATCC 13048)
Enterobacter cloacae	(ATCC 13047)
Enterococcus faecalis	(ATCC 29212)

Escherichia coli	(ATCC 25922)
Klebsiella pneumoniae	(ATCC 13883)
Peptostreptococcus anaerobius	(ATCC 27337)
Proteus vulgaris	(ATCC 8427)
Pseudomonas aeruginosa	(ATCC 10145)
Salmonella enterica Serovar enteritidis	(ATCC 13076)
Salmonella enterica Serovar typhimurium	(ATCC 14028)
Shigella flexneri	(ATCC 12022)
Shigella sonnei	(ATCC 25931)
Staphylococcus aureus	(ATCC 25923)
Staphylococcus epidermidis	(ATCC 12228)
Vibrio parahaemolyticus	(ATCC 17802)

The *C. sordellii* strain ATCC 9714 did not cross react in the *Serazym*® Clostridium difficile Toxin A+B although some publications describe cross reactivities of toxins of some *C. sordellii* strains with anti-C. difficile toxin antibodies.

Common Advices And Precautions

This kit is for *in-vitro* use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only. Do not use reagents from damaged packages or bottles. The expiration dates stated on the respective labels are to be observed. **Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.**
The sample diluent, wash buffer, TMB/substrate solution and stop solution is universally applicable for the *Serazym*® stool ELISA Adenovirus (E-017), Rotavirus (E-020), Astrovirus (E-045), Norovirus (E-061), Clostridium difficile Toxin A+B (E-040), Clostridium difficile GDH (E-107), Campylobacter (E-093), H. pylori 2nd Gen. (E-114), Entamoeba histolytica (E-018), Cryptosporidium parvum (E-039), Giardia lamblia (E-038) and Giardia (E-106).
Do not use reagents from other manufacturers. Avoid time shift during dispensing of reagents. All reagents should be kept at 2…8°C before use. Some of the reagents may contain biocides as preservative. Further information can be found in the safety data sheet. They must not be swallowed or allowed to come into contact with skin or mucous membranes. Handle all components and all patient samples as if potentially hazardous. Since the kit contains potentially hazardous materials, the following precautions should generally be observed:

Do not smoke, eat or drink while handling kit material!
Always use protective gloves!
Never pipette material by mouth!
Note safety precautions of the single test components!

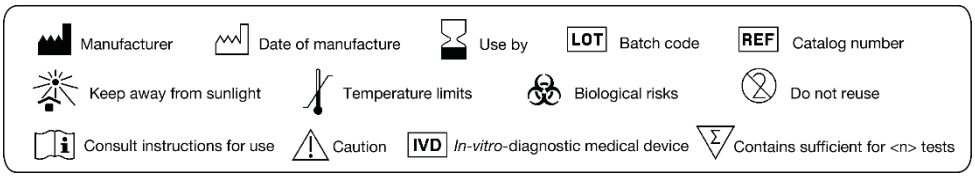
History Of Changes

Version	Section	Modifications
2020-07-21	Intended Use	Correction
	Test Components	Correction
	Preparation And Storage Of Samples	Update
	Assay Procedure	Update
	Common Advices And Precautions	Update
	History of Changes	New section “History of Changes“

Incubation Scheme *Serazym*® Clostridium difficile Toxin A+B (E-040)

1.	pipette	
	100 µl	CONTROL + *(4)*
	100 µl	CONTROL – *(5)*
	100 µl	**diluted stool specimen** or **culture suspension**
	60 min	incubation (RT) **alternatively 30 min while shaking**
	5 x wash	with wash solution
2.	3 drops (or 120 µl)	CONJ BIOTIN *(6/1)*
	30 min	incubation (RT) **alternatively 15 min while shaking**
	5 x wash	with wash solution
3.	3 drops (or 120 µl)	CONJ STREPT *(6/2)*
	30 min	incubation (RT) **alternatively 15 min while shaking**
	5 x wash	with wash solution
4.	3 drops (or 120 µl)	SUBSTR TMB *(7)*
	15 min	incubation protected from light (RT) **without shaking**
5.	3 drops (or 120 µl)	STOP *(8)*

Read OD at 450 / ≥ 620 nm



Serazym ® Clostridium difficile GDH

Enzyme immunoassay for detection of *Clostridium difficile* GDH in faecal specimens

REF E-107 96 REF E-107-A2 2x 96 IVD *In-vitro*-diagnostic medical device CE

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Introduction

The spore forming anaerobic bacterium *Clostridium difficile* is the most common cause of nosocomial diarrheas predominantly developing in the course of antibiotic therapy. The severity of the disease in dependence of the health status of the patient but also of the pathogenicity of the *C. difficile* strain may range from bloody diarrhea to Colitis and pseudomembranous Colitis up to toxic megacolon. The two exotoxins A and B are responsible for the development of the symptoms. Most *Clostridium difficile* strains produce both, toxin A and B, but some are characterized by isolated toxin B production. Atoxigenic strains are generally considered as nonpathogenic and therefore diagnostically irrelevant. The *Clostridium difficile* specific enzyme Glutamatdehydrogenase, also described as “common antigen” is produced by both: toxigenic and atoxigenic strains.
The human intestine is colonized with *Clostridium difficile* in 2 - 3% of healthy adults and in up to 50% of children < 2 years of age. Therefore, the diagnosis of a *Clostridium difficile* associated diarrhea (CDAD) has to be confirmed by the detection of the toxins A and B.
Changes in the epidemiological situation are observed since several years: Increasingly also young adults and not hospitalized patients get sick developing the symptoms of CDAD. An increasing number of severe developments of the disease caused by highly virulent strains also occur. In this context testing of patient´s status as carrier seems reasonable.
Different approaches have developed in laboratory diagnosis of *C. difficile* infections. Usually direct toxin A and B detection from stool or enrichment cultures is performed by enzyme immunoassay. In addition, a new two-step procedure has been established in several laboratories that comprise a first screening of *C. difficile* GDH and the subsequent testing for toxins A and B of GDH positive samples. The isolated detection of GDH is neither sufficient for diagnosing a CDAD nor suitable for a therapeutic decision, but always needs to be completed by the testing for toxins A and B.
References:

1. Wilkins TD and Lyerly DM (2003): „Clostridium difficile Testing: after 20 Years, Still Challenging“ Journal Of Clinical Microbiology, Vol. 41, No.2, p531-534
2. Von Eichel-Streiber C und Braun V (2008): “Das difficile Clostridium” Journal Of Laboratory Medicine, Vol. 32, No. 4, p219-234
3. Lyerly DM, Barroso LA, Wilkins TD (1991): “Identification of the latex test-reactive protein of Clostridium difficile as glutamate dehydrogenase.” Journal Of Clinical Microbiology, Vol. 29, p2639-2642.
4. Zhen L, Keller SF, Lyerly DM et al. (2004): „Multicenter Evaluation of a New Screening Test That Detects Clostridium difficile in Faecal Specimens“ Journal Of Clinical Microbiology, Vol. 42, No. 8, p3837-3840.
5. Peterson LR and Robicsek A (2009): “Does My Patient Have Clostridium difficile Infection?” Annals of Internal Medicine, Vol. 151, No. 3, p176-180.

Intended Use

***Serazym*® Clostridium difficile GDH is an *in-vitro* diagnostic medical device for direct detection of Glutamatdehydrogenase (GDH) of *Clostridium difficile* in faecal specimens.**

Principle of the Test

Serazym® Clostridium difficile GDH is a one-step enzyme immunoassay on the basis of polyclonal and monoclonal antibodies to Glutamatdehydrogenase (GDH) of *Clostridium difficile*. Diluted stool specimens and monoclonal anti-GDH antibodies are dispensed simultaneously into the wells of a microtitration plate coated with polyclonal anti-GDH antibodies. After an incubation time of 60 min at room temperature (RT) unbound components are removed by a washing step. Alternatively, the first incubation can be shortened to 30 min by shaking. HRP converts the subsequently added colourless substrate solution of 3,3’,5,5’-Tetramethylbenzidine (TMB) within a 10 min reaction time into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells turning the solution from blue to yellow. The optical density (OD) of the solution read at 450 / $\geq$ 620 nm is directly proportional to the specifically bound amount of GDH antigen in the respective sample.
Considering the cut-off value results are interpreted as positive or negative.

Test Components

			For 96 Wells	For 2x 96 Wells
1	WELLS	**Microtitration plate** coated with polyclonal anti-C.difficile GDH antibodies (sheep)	12 single breakable 8-well strips colour coding green vacuum-sealed with desiccant	2x 12 single breakable 8-well strips colour coding green vacuum-sealed with desiccant
2	WASHBUF CONC 10x	**Wash buffer** 10-fold	100 ml concentrate for 1000 ml solution white cap	2x 100 ml concentrate for 2x 1000 ml solution white cap
3	DIL	**Sample diluent**	100 ml · ready to use coloured yellow black cap	2x 100 ml · ready to use coloured yellow black cap
4	CONTROL +	**Positive control** Recombinant *C. difficile* GDH	2.0 ml · ready to use coloured blue red cap	4.0 ml · ready to use coloured blue red cap
5	CONTROL –	**Negative control** *C. difficile* GDH negative sample	2.0 ml · ready to use coloured blue green cap	4.0 ml · ready to use coloured blue green cap
6	CONJ HRP	**HRP-conjugate** HRP-labelled, monoclonal anti-C.difficile GDH antibodies (mouse)	15 ml · ready to use coloured green brown cap	25 ml · ready to use coloured green brown cap
7	SUBSTR TMB	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml · ready to use blue cap	28 ml · ready to use blue cap
8	STOP	**Stop solution** 0.25 M sulphuric acid	15 ml · ready to use yellow cap	28 ml · ready to use yellow cap

Preparation and Storage of Samples

Collection and storage

Stool samples should be stored at 2…8°C immediately after collection and processed within 72 hours. Longer storage is possible at least at-20°C. Repeated (> 3x) freezing and thawing of samples should be avoided. Stool samples already diluted with the *Serazym*® sample diluent can be stored for up to 72 h at 2…8 °C before testing in the ELISA.

Preparation

Mix samples thoroughly. Pipette 1000 µl of sample diluent into a clean tube. Using a disposable stirring rod tansfer about 200 mg (diameter about 4 - 6 mm) of faeces if solid or pipette 200 µl if liquid into the tube and suspend thoroughly. Spin down floating particles in a micro centrifuge at maximum speed for 1 min if necessary.

Materials required but not provided

Micropipettes · multi-channel pipette or multi-pipette · Reagent container for multi-channel pipette · 8-channel wash comb with vacuum pump and waste bottle or microplate washer or 8-channel pipette · microplate reader with optical filters for 450 nm for measurement and ≥ 620 nm for reference · distilled or deionized water · glassware · tubes (2 ml) for sample preparation · orbital shaker for performance of test variant 2

Preparation and Storage of Reagents

Kit size and expiry
One kit is designed for 1x 96 or 2x 96 determinations. The expiry date of each component is reported on its respective label, that of the complete kit on the outer box label. Upon receipt, all test components have to be kept at 2…8°C, preferably in the original kit box. After opening all kit components are stable for at least 2 months, provided proper storage. The ready to use wash solution can be used for at least one month when stored at 2…8°C.

Reagent preparation
Allow all components to reach room temperature prior to use in the assay. The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.
Prepare a sufficient amount of wash solution by diluting the 10-fold concentrated wash buffer 1 + 9 with distilled or deionized water.
For Example: 10 ml wash buffer concentrate *(2)* + 90 ml distilled or deionized water.

Assay Procedure

The *Serazym*® Clostridium difficile GDH can be performed in two ways:

1. Incubation without shaking; complete test duration 1 h and 10 minutes
2. Incubation with shaking; complete test duration 40 minutes

Dilute samples with sample diluent *(3)* 1 : 6 e.g. 200 mg or 200 µl stool + 1.0 ml sample diluent *(3)* and mix thoroughly.
Avoid any time shift during dispensing of reagents and samples.
Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle and that residual fluid is completely drained in every single wash cycle!
Avoid direct light exposure of the TMB substrate solution!

Working steps variant 1: without shaking

1. Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.
2. Dispense 3 drops (or 100 µl) **CONJ HRP** HRP-conjugate *(6)* per well and
3. Pipette: 100 µl **CONTROL +** positive control *(4)*
   100 µl **CONTROL –** negative control *(5)*
   100 µl **diluted sample**, mix gently
4. Cover plate and incubate for 60 min at RT.
5. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
6. Dispense 3 drops (or 100 µl) **SUBSTR TMB** substrate *(7)* per well.
7. Incubate for 10 min at RT protected from light.
8. Dispense 3 drops (or 100 µl) **STOP** stop solution *(8)* per well, mix gently.
9. Read OD at 450 nm / ≥ 620 nm with a microplate reader within 30 min after reaction stop.

Working steps variant 2: with shaking

1. Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.
2. Dispense 3 drops (or 100 µl) **CONJ HRP** HRP-conjugate *(6)* per well and
3. Pipette: 100 µl **CONTROL +** positive control *(4)*
   100 µl **CONTROL –** negative control *(5)*
   100 µl **diluted sample**, mix gently
4. Cover plate and incubate for 30 min at RT on an orbital shaker with a frequency of 500 – 700 / min.
5. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
6. Dispense 3 drops (or 100 µl) **SUBSTR TMB** substrate *(7)* per well.
7. Incubate for 10 min at RT protected from light.
8. Dispense 3 drops (or 100 µl) **STOP** stop solution *(8)* per well, mix gently.
9. Read OD at 450 nm / ≥ 620 nm with a microplate reader within 30 min after reaction stop.

Result Interpretation

Qualitative evaluation
Cut-off determination: OD negative control + 0.10
Samples with OD values equal to or higher than the cut-off value are considered positive, samples with OD values below the cut-off value are considered negative in the *Serazym*® C. difficile GDH.

Reference Values

***Serazym*® Clostridium difficile GDH**	
Positive	≥ Cut-off
Negative	< Cut-off

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters too. Therefore, the mentioned reference values provide a guide only to values which might be expected.

Test validity
The test run is valid if:

- the mean OD of the negative control is ≤ 0.20 (manual performance)
  ≤ 0.30 (automatic performance)
- the mean OD of the positive control is ≥ 1.00

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

Limitations of the procedure

The qualitative determination of *Clostridium difficile* GDH in stool specimens by enzyme immunoassay is not equivalent to the diagnosis of a *C. difficile* associated disease (CDAD). CDADs are caused by the toxins A and B of pathogenic *C. difficile* strains. Therefore, a positive GDH test result has to be supplemented by testing for toxins A and B, in order to confirm or exclude a toxigenic strain. On the other hand a negative GDH test result does not necessarily exclude a *C. difficile* infection. After dilution in sample diluent the samples should be tested by ELISA as quickly as possible, at least within 72 hours, because storage-depending antigen degradation may cause false negative results. More than 3 freeze-thaw cycles may also cause false negative results due to antigen degradation. As a result of inhomogeneous antigen distribution in some stool samples insufficient homogenizing may also cause false negative results. Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions or solid particles after centrifugation of the suspension can cause false negative as well as false positive results. The overall interpretation of the ELISA results should always consider the microbiological examination as well as clinical findings.

Automatic Processing

Performing the *Serazym*® Clostridium difficile GDH on fully automated microplate processors (e.g. DS2 or DSX) may cause elevated absorbances in comparison to the manual procedure due to individual differences concerning wash procedures and general technical specifications of the equipment. In these cases a maximum value of 0.3 absorbance units is permissible for the negative control. It is recommended to use a wash procedure including 10 seconds soak time per strip and wash step followed by a wash step with distilled or deionized water with 10 seconds of soak time after the final wash step of each wash cycle. If necessary the number of washing steps can be enhanced from 5x to 7x - 8x.

Correlation: manual – automatic processing

A panel of 90 stool specimens (45 positive and 45 negative samples) was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with r = 0.999.

Performance Characteristics

Precision

Intra-assay coefficient of variation (CV) calculated from 8-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	2.800	0.040	1.52
2	1.960	0.045	2.46
3	0.611	0.041	7.19
4	0.352	0.017	5.15

Inter-assay coefficient of variation (CV) in 5 different test runs calculated from twofold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	1.707	0.250	14.6
2	1.155	0.148	12.8
3	0.895	0.098	10.9
4	0.381	0.068	17.9

Lot-to-Lot reproducibility in 3 different production lots calculated from 8-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	1.840	0.166	9.0
2	1.285	0.172	13.4
3	0.349	0.093	26.6
4	0.026	0.005	17.6

Lower detection limit
The lower detection limit of Glutamatdehydrogenase (GDH) in the *Serazym*® Clostridium difficile GDH was determined 10 ng/ml by titration of recombinant GDH antigen.

Sensitivity and specificity

Sensitivity in comparison to PCR
Ninety eight out of 102 stool specimens characterized *Clostridium difficile* positive by PCR were tested positive with the *Serazym*® ELISA corresponding to a sensitivity of 96.1%.

Comparative sensitivity and specificity
In two comparative studies 235 and 170 stool samples were tested in parallel in the *Serazym*® Clostridium difficile GDH and 2 other commercially available ELISAs respectively.

Study 1

n = 235	comparative ELISA 1 positive	comparative ELISA 1 negative
***Serazym*® ELISA positive**	101	3**
***Serazym*® ELISA negative**	12*	119

Sensitivity: 89.4% · Specificity: 97.5%

* 10 out of 12 *Serazym*® ELISA negative and comparative ELISA 1 positive samples were tested negative in 2 other commercial ELISAs. Sensitivity amended: 98.1%
** One sample was confirmed true positive by PCR. Specificity amended: 98.3%

Study 2

n = 170	comparative ELISA 2 positive	comparative ELISA 2 negative
***Serazym*® ELISA positive**	69	1*
***Serazym*® ELISA negative**	3	97

Sensitivity: 95.8% · Specificity: 98.9%

* This sample was confirmed true positive by PCR.
Specificity amended: 100%

Cross reactivity
Negative stool specimens have been spiked with $\geq 10^8$ colony forming units of the following microorganisms and tested negative with the *Serazym*® ELISA (OD 450 / 620 nm < Cut-Off):

Aeromonas hydrophila	(ATCC 7966)
Bacillus cereus	(ATCC 11778)
Bacillus subtilis	(ATCC 6633)
Bacteroides fragilis	(ATCC 25285)
Candida albicans	(ATCC 10231)
Campylobacter coli	(ATCC 33559)
Campylobacter jejuni	(ATCC 33291)
Citrobacter freundii	(ATCC 8090)
Clostridium sordellii	(ATCC 9714)
Enterobacter aerogenes	(ATCC 13048)
Enterobacter cloacae	(ATCC 13047)
Enterococcus faecalis	(ATCC 29212)
Escherichia coli	(ATCC 25922)

Klebsiella pneumoniae	(ATCC 13883)
Peptostreptococcus anaerobius	(ATCC 27337)
Proteus vulgaris	(ATCC 8427)
Pseudomonas aeruginosa	(ATCC 10145)
Salmonella enterica Serovar enteritidis	(ATCC 13076)
Salmonella enterica Serovar typhimurium	(ATCC 14028)
Shigella flexneri	(ATCC 12022)
Shigella sonnei	(ATCC 25931)
Staphylococcus aureus	(ATCC 25923)
Staphylococcus epidermidis	(ATCC 12228)
Vibrio parahaemolyticus	(ATCC 17802)
Vibrio cholerae	Clinical isolate
Yersinia enterocolitica Serotyp 03, 09	Clinical isolates

Interference
None of the following substances added to GDH positive and negative stool samples showed a significant impact on the test result:
barium sulphate (5%), Buscopan® (2 mg/ml), cyclamate (5%), Diclofenac (2 mg/ml), haemoglobine (5 mg/ml), Hylak® N (5%), Immodium® akut duo (0.2/12.5 mg/ml), Iberogast® (5%), loperamide (0.2 mg/ml), metronidazole (2 mg/ml), mucin (5 mg/ml), Nexium® (2 mg/ml), palmitic acid (20%), Pentofuryl® (2 mg/ml), Pepto-Bismol (1 mg/ml), Perenterol (2.5 mg/ml), Rennie® (8 mg/ml), Simagel® (2 mg/ml), stearic acid (20%), vancomycin hydrochloride (0.5%).

Common Advices and Precautions

This kit is for *in-vitro* use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only. Do not use reagents from damaged packages or bottles. The expiration dates stated on the respective labels are to be observed. **Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.**
The sample diluent, wash buffer, TMB/substrate solution and stop solution is universally applicable for the *Serazym*® stool ELISA Adenovirus (E-017), Rotavirus (E-020), Astrovirus (E-045), Norovirus (E-061), Clostridium difficile Toxin A+B (E-040), Clostridium difficile GDH (E-107), Campylobacter (E-093), H. pylori 2nd Gen. (E-114), Entamoeba histolytica (E-018), Cryptosporidium parvum (E-039), Giardia lamblia (E-038) and Giardia (E-106).
Do not use reagents from other manufacturers. Avoid time shift during dispensing of reagents. All reagents should be kept at 2…8°C before use. Some of the reagents may contain biocides as preservative. Further information can be found in the safety data sheet. They must not be swallowed or allowed to come into contact with skin or mucous membranes. Handle all components and all patient samples as if potentially hazardous. Since the kit contains potentially hazardous materials, the following precautions should generally be observed:

Do not smoke, eat or drink while handling kit material!
Always use protective gloves!
Never pipette material by mouth!
Note safety precautions of the single test components!

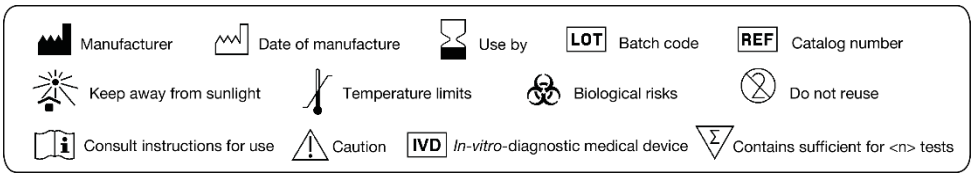
History of Changes

Version	Section	Modifications
2021-02-02	Preparation And Storage Of Samples, Assay Procedure	Update

Incubation Scheme *Serazym*® Clostridium difficile GDH (E-107)

1.		3 drops (or 100 µl)	**CONJ HRP** *(6)*
		+	
		pipette	
		100 µl	**CONTROL +** *(4)*
		100 µl	**CONTROL –** *(5)*
		100 µl	**stool sample**, mix gently
		60 min	incubation (room temperature) **alternatively 30 min while shaking**
		5x wash	with wash solution
2.		3 drops (or 100 µl)	**SUBSTR TMB** *(7)*
		10 min	incubation protected from light (room temperature) **without shaking**
3.		3 drops (or 100 µl)	**STOP** *(8)*

Read OD at 450 / ≥ 620 nm



NovaTec Immundiagnostica GmbH

NovaLisa®

Clostridium tetani toxin IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: TETG0430 (96 Determinations)

ENGLISH

1. INTRODUCTION

Clostridia are spore-forming gram-positive bacteria. The round spores are build at the terminal end which results in the microscope in a “tennis racket” like shape.

Tetanus develops only when spores of Clostridium tetani germinate under strict anaerobic conditions after gaining access to wounds and small lacerations. The clinical manifestation of the disease is primary not caused by the invasion of the exciter but by the secretion of a powerful neurotoxin (tetanospasmin). This toxin blocks the inhibition of the signal transduction and has a high affinity to the central nervous system. The consequence is hyper excitability of the muscles to external stimuli in combination with a principal increase of the muscle tonus without influence of consciousness. It starts with tonic spasm of muscles (trimus), mimic muscles and gallet muscles. Neck, back and abdominal musculature follow. At the same time the appearance of refectory spasm of whole muscle groups can hamper breathing. Hyper salivation and swallowing problems cause aspiration and pneumonia with the next breath.

Clostridium tetani is ubiquitous present in soil and intestine of humans and animals. Ingestion of bacteria or growth in the intestine of man or animal is without harm. The spores are extremely resistant towards heat and can stay infectious for a long period. The bacteria can get under the skin by even smallest wounds. In Europe tetanus mainly occurs after injuries and sometimes postoperative whereas in developing countries Tetanus is widely disseminated. The WHO assumes that one million people die because of tetanus worldwide per year.

Tetanus toxin is an excellent immunogen in man - only one antigenic type of toxin. The only effective way to control tetanus is by prophylactic active immunization.

Species	**Disease**	**Symptoms (e.g.)**	**Transmission route**
Clostridium tetani	Tetanus	Trismus, dysphagia, severe, painful spasms of whole muscle groups, hypersalivation, aspiration, asphyxia	Injury (Infection of the wound with Clostridium tetani)

The presence of pathogen or infection may be identified by

- Microscopy
- Serology: e.g. ELISA

2. INTENDED USE

The Clostridium tetani toxin IgG ELISA is intended for the quantitative determination of IgG class antibodies against Clostridium tetani 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.
Microplates 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 microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Clostridium tetani toxin Coated Microplate (IgG):** 12 break apart 8-well snap-off strips coated with Clostridium tetani toxin (toxoid) antigens; in resealable aluminium foil.
- **IgG Sample Diluent:** 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.
- **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.
- **Clostridium tetani toxin anti-IgG 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; < 5% NMP.
- **Clostridium tetani toxin IgG Standards:** 4 vials, each containing 2 ml standard (human serum or plasma); coloured yellow; ready to use.

  Standard A: 0.0 IU/ml; blue cap

  Standard B: 0.1 IU/ml; green cap

  Standard C: 0.5 IU/ml; yellow cap

  Standard D: 1.0 IU/ml; red cap

  The standards are calibrated in accordance with the Who International Standard; ”1st International Standard for Tetanus Immunoglobulin, Human”; NIBSC Code: TE-3.

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 microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- 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. Coated Microplate

The break-apart snap-off strips are coated with Clostridium tetani 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. For CSF please use the instruction for use ABVL0001. 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 Diluent. Dispense 10 µl sample and 1 ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. Test Preparation

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 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.

8.2. Measurement

Adjust the ELISA microwell plate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA microwell plate 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 to be considered valid, the following criteria must be met:

- **Substrat-Blank: Absorbance value < 0.100**
- **Standard A: Absorbance value < 0.200**
- **Standard B: Absorbance value > 0.150**
- **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.

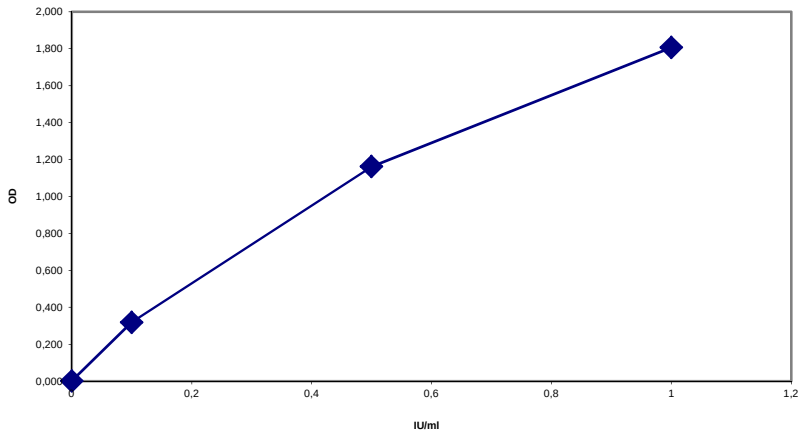
9.2. Calculation of Results

In order to obtain **quantitative results in IU/ml** blot the (mean) absorbance values of the 4 Standards A - D on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0.0 / 0.1 / 0.5 and 1.0 IU/ml) and draw a standard 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 and Recommendations [IU/ml]

< 0.1 IU/ml	No protective antibody level or no reliable protection! Immediate full course of basic immunization or booster injection and control of antibody concentration 4 to 6 weeks later is recommended.
0.11 - 0.5 IU/ml	Reliable protection! Booster injection and control of antibody concentration 4 to 6 weeks later is recommended.
0.51 - 1.0 IU/ml	Reliable protection; control of antibody concentration after about 2 years is recommended. Booster injection is not required. Note: In cases of antibody concentrations greater than 0.5 IU/ml vaccination can cause side effects!
1.1 - 5.0 IU/ml	Range of long term protection: Control after 5 to 10 years
> 5.0 IU/ml	Range of long term protection: Control after 10 years 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 (E)	Cv (%)
#1	24	1.306	3.60
#2	24	1.805	3.46
#3	24	1.591	5.34
Interassay	**n**	**Mean (IU/ml)**	**Cv (%)**
#1	12	0.060	9.62
#2	12	0.084	11.33
#3	12	0.658	13.99

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 100.0% (95% confidence interval: 76.84% - 100.0%).

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 99.22% (95% confidence interval: 95.76% - 99.98%).

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 0.01 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 0.01 IU/ml – 1 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

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. 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 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/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 who are familiar with good laboratory practice.

12.1. 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.: TETG0430 Clostridium tetani toxin IgG ELISA (96 Determinations)