



















Serazym[®] Clostridium difficile Toxin A+B

Enzyme immunoassay for the qualitative determination of *Clostridioides difficile* specific enterotoxin TcdA (toxin A) and cytotoxin TcdB (toxin B) in stool samples of human origin or in culture suspensions

REF	E-040		96
IVD	In-vitro-diagnostic medical device		CE



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 In-vitro diagnostic medical device	 Unique device identifier	 Manufacturer
 Country of manufacture and date of manufacture	 Article number	 Serial number
 Keep away from sunlight	 Humidity limitation	 Batch code
 Consult instructions for use	 Temperature limit	 Do not reuse
 Sufficient for <i>n</i> tests	 Biohazard	 Use-by date
		 Attention

Intended Use

Serazym® Clostridium difficile Toxin A+B is an IVD test for the qualitative determination of *Clostridioides* (former: *Clostridium*) *difficile* specific enterotoxin TcdA (toxin A) and cytotoxin TcdB (toxin B) in stool samples of human origin or in culture suspensions through manual or semi-automatic processing by a laboratory professional user.

It is intended to aid in the diagnosis of a *C. difficile* infection (CDI) in specimen materials from patients with symptoms of a *C. difficile*-associated gastroenteritis.

Principle of the Test

Serazym® Clostridium difficile Toxin A+B is an enzyme immunoassay based on monoclonal antibodies against *Clostridioides* (formerly: *Clostridium*) *difficile* toxins A and B. Diluted, untreated stool samples or culture suspension as well as negative and positive control samples are dispensed into wells of the microtiter plate coated with monoclonal anti-toxin A and anti-toxin B antibodies. After incubation, unbound components are removed by a washing step, then biotinylated monoclonal anti-toxin A and anti-toxin B antibodies are dispensed into the wells. After incubation, unbound components are removed by a washing step. The bound biotinylated antibodies react with horseradish peroxidase (HRP)-conjugated streptavidin in a further incubation step. After incubation and a washing step, HRP converts the colorless substrate solution to a blue reaction product in the following enzymatic reaction step. The reaction is stopped by addition of the stop solution, resulting in a color change from blue to yellow. The optical density (OD) of the reaction product measured at 450 nm measuring filter and ≥ 620 nm reference filter, respectively, is directly proportional to the concentration of specifically bound *C. difficile* toxin A and B antigens.

Test Components (Delivery Scope)

			For 96 wells
1	WELLS	Microtiter plate Coated with < 15 µg/mL monoclonal anti-toxin A and anti-toxin B antibodies (mouse)	12 single breakable 8-well strips, red color marking, vacuum-sealed with desiccant
2	WASHBUF (10x)	Wash buffer (10x) Seramun® Wash buffer A TRIS-based buffer	100 mL concentrate for 1000 mL solution, colorless white cap
3	DIL	Sample diluent Seramun® Sample diluent A Phosphate-based buffer	100 mL, ready to use, colored yellow, black cap
4	CONTROL +	Positive control < 5 µg/mL recombinant <i>C. difficile</i> antigen	2.0 mL, ready to use, colored blue, red cap
5	CONTROL -	Negative control TRIS-based buffer	2.0 mL, ready to use, colored blue, green cap
6/1	CONJ BIOTIN	Biotin conjugate < 1 µg/mL biotinylated, monoclonal anti-toxin A and anti-toxin B antibodies (mouse)	15 mL, ready to use, colored green, white cap
6/2	CONJ STREPT	Streptavidin- HRP conjugate < 1µg/mL	15 mL, ready to use, colored red, green cap
7	SUBSTR	Substrate SeramunBlau® automat fast < 0.1 % 3,3',5,5'-tetramethylbenzidine; < 0.05 % hydrogen peroxide	15 mL, ready to use, colorless, blue cap

8	STOP	Stop solution SeramunBlau® stop 0.25 M sulphuric acid	15 mL, ready to use, colorless, yellow cap
9		Certificate of Analysis	1 piece
10		Instructions for Use	1 piece

Additional Materials and Aids Required for the Test Procedure

Adjustable single-channel micropipette • 8-channel micropipette or multi-channel micropipette with pipette tips • reagent reservoirs for multi-channel pipetting • 8-channel wash comb with vacuum pump and waste bottle or microplate washer • microplate reader with 450 nm measuring filter and ≥ 620 nm reference filter • deionized water • measuring cylinder • tubes for sample preparation • orbital shaker for assay procedure with shaker

Important Information



This device is for *in-vitro* diagnostic use only. Follow the instructions carefully. The kit may be used by health professionals only.

Do not use reagents from damaged packages or bottles. The shelf life specified must be observed. Do not mix components with reagents from other manufacturers.

Mixing of test kit components of different lots is permitted only for sample diluent, wash buffer (10x), negative control, substrate and stop solution.

The wash buffer (10x), sample diluent, negative control, substrate, and stop solution are universally applicable for Serazym® stool ELISA Adenovirus (E-017), Astrovirus (E-045), Norovirus (E-061), Rotavirus (E-020), Campylobacter (E-093), Clostridium difficile GDH (E-107), Clostridium difficile Toxin A+B (E-040), Cryptosporidium parvum (E-039), Entamoeba histolytica (E-018), Giardia (E-106) and H. pylori 2nd Gen. (E-114).

All serious incidents occurring in relation with Serazym® Clostridium difficile Toxin A+B must be reported to the manufacturer and the competent authority of the EU member state in which user and/or patient are located.

Information on Assay Procedure

All reagents should be stored at 2...8 °C. Bring all test components to room temperature before use. Reagents that appear contaminated should not be used.

Each well of a microtiter plate can be used once only. Each sample and control have to be pipetted with a new pipette tip. Positive and negative controls are ready to use.

For larger sample series, pipetting reagents from liquid reservoirs using a multi-channel micropipette is recommended to avoid time delays and contaminations. Follow the pipetting scheme and time schedules of the protocol.



The aspiration and washing steps can be performed manually or with the help of a microplate washer or waterjet pump. Allow the wash buffer to remain in the wells for at least 5 seconds per wash cycle. Remove wash buffer residues by thoroughly aspirating or tapping out the wells!

Protect the substrate from light!

Safety Instructions

Reagents must not be swallowed. Contact with skin or mucous membranes should be avoided. Handle all components and patient samples as if potentially hazardous and infectious. Additional information may be taken from the Safety Data Sheet.

Product contains the following hazardous component/-s:

Test component	Hazard labeling and supplementary information on ingredients
WELLS	Contains material of animal origin.
WASHBUF (10x)	<p>EUH208: Contains reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1). May produce an allergic reaction.</p> <p>EUH210: Safety data sheet available on request.</p> <p>Preservatives: < 0.0015 % reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1); < 0.1 % 5-bromo-5-nitro-1,3-dioxane</p>
DIL	<p>Contains material of animal origin.</p> <p>Preservatives: < 0.1 % sodium azide</p>
CONTROL +	<p>Contains material of microbial and animal origin.</p> <p>Preservatives: < 0.1 % sodium azide</p>
CONTROL -	<p>Contains material of animal origin.</p> <p>Preservatives: < 0.01 % sodium azide</p>
CONJ BIOTIN	<p>EUH208: Contains reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1). May produce an allergic reaction.</p> <p>EUH210: Safety data sheet available on request.</p> <p>Contains material of microbial and animal origin.</p> <p>Preservative: < 0.0015 % reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1); < 0.1 % 5-bromo-5-nitro-1,3-dioxane</p>
CONJ STREPT	<p>EUH210: Safety data sheet available on request.</p> <p>Contains material of animal origin.</p> <p>Preservative: < 0.00015 % reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1); < 0.1 % 5-bromo-5-nitro-1,3-dioxane</p>
SUBSTR	<p>Hazard component: 2-pyrrolidone</p> <p>Signal word: Danger</p> <div style="display: flex; justify-content: center; align-items: center; gap: 10px;">   </div> <p>H360: May damage fertility or the unborn child.</p> <p>P201: Obtain special instructions before use.</p> <p>P280: Wear protective gloves/protective clothing/eye protection/face protection.</p> <p>P308+P313: IF exposed or concerned: Get medical advice/attention.</p> <p>Restricted to professional users.</p> <p>Preservatives: < 0.00015 % reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)</p>
STOP	-

Limitations of the Procedure

The qualitative enzyme immunological detection of toxin A and B of *Clostridioides difficile* in stool samples does not allow a correlation between the measured OD and the severity of an infection. Also, it is not allowed to correlate absorbances of samples with the absorbance of the positive control. Cross contamination of reagents and samples may result in false results. Incorrect dilutions, insufficiently homogenized samples, and particles not sedimented by centrifugation may cause false positive as well as false negative test results. Formalin-treated samples may cause false positive results and must therefore not be used in the test. A negative test result obtained with Serazym® Clostridium difficile Toxin A+B does not exclude an infection. False negative tests may result from improper timing of sample collection or inhomogeneous antigen distribution in the sample. The overall interpretation of the ELISA test result should consider the full clinical picture. Individual cases may require retesting at intervals of several weeks.

Sample Treatment

Sample Collection

Collect stool sample in suitable sampling container.

Example: Stool collection tube, with spoon, screw cap, (LxØ): 107 x 25 mm, transparent

Sample Shelf Life and Storage

Stool samples should be stored immediately after collection at 2...8 °C or -20 °C and examined within 72 h. Repeated (> 3x) freezing and thawing of samples should be avoided due to the risk of incorrect results. Stool samples that have already been diluted in Seramun® Sample diluent A according to the instructions for use can be stored at 2...8 °C for 72 h and subsequently analyzed by ELISA.

Sample Preparation

Mix untreated stool samples well and dilute 1 : 6 with sample buffer.

Example: Pipette 500 µL sample buffer into a reaction tube. For solid or semi-solid stool samples transfer 100 mg (approx. 2 – 3 mm diameter) with a disposable stick, for liquid stool samples transfer 100 µL into the sample buffer and mix thoroughly. If necessary, sediment suspended particles by centrifugation in a microcentrifuge for 1 min at maximum speed.

Reagent Treatment

Reagent Shelf Life and Storage

The complete test kit with sealed reagent bottles and microtitration strips can be stored at 2...8 °C until the printed expiration date. All opened test kit components are stable for up to 2 months when stored properly at 2...8 °C. The diluted wash buffer can be stored at 2...8 °C for up to 1 month.

Reagent Preparation

Microtiter plate with breakable 8-well strips is vacuum sealed with desiccant. Allow packaging to reach room temperature before opening. Protect unused wells from moisture and store carefully refrigerated with desiccant in the original bag. Dilute wash buffer (10x) 1 : 10 with deionized water.

Example: 10 mL Seramun® Wash buffer A (10x) + 90 mL deionized water.

Assay Procedure

1. Allow test reagents and required number of wells to reach room temperature (RT). Shake reagents gently before use. Avoid foaming.
2. Pipette 100 μ L **CONTROL +** Positive control
100 μ L **CONTROL -** Negative control
100 μ L diluted stool specimen or culture suspension.
3. Cover the plate and incubate for 60 min at RT.
4. Decant, then wash each well 5x with 300 μ L diluted wash buffer. Tap the plate dry on absorbent paper if necessary.
5. Add 120 μ L **CONJ BIOTIN** biotin conjugate per well.
6. Cover the plate and incubate for 30 min at RT.
7. Decant, then wash each well 5x with 300 μ L diluted wash buffer. Tap dry onto absorbent paper if necessary.
8. Add 120 μ L **CONJ STREPT** streptavidin-HRP conjugate per well.
9. Cover plate and incubate for 30 min at RT.
10. Decant, then wash each well 5x with 300 μ L diluted wash buffer. Tap the plate dry on absorbent paper if necessary.
11. Add 120 μ L **SUBSTR** substrate per well.
12. Incubate for 15 min at RT **protected from light**.
13. Add 120 μ L **STOP** stop solution per well, mix gently.
14. Read OD at 450 nm measuring filter and \geq 620 nm reference filter with a microplate reader within 30 min following reaction stop.

Evaluation of Results

Qualitative Evaluation

Cut-off determination: OD negative control +0.20

Samples showing OD values equal with or higher than the cut-off are considered positive, samples with OD values 10 % below cut-off are considered negative for *C. difficile* toxin A and B antigens. Samples showing OD values within 10 % below the cut-off and cut-off have to be considered borderline and should be tested again. In case of a repeated borderline result a second stool sample of the respective patient should be analyzed.

The test run is valid if:

- the mean OD value of the negative control is \leq 0.20 (manual processing)
 \leq 0.30 (automatic processing)
- the mean OD value of the positive control is \geq 1.00

If the above-mentioned quality criteria are not met, test should be repeated strictly following the test procedure (incubation times and temperatures, sample and wash buffer dilution, wash steps, etc.). In case of repeated failure of the quality criteria contact the manufacturer.

Interpretation of Results

Positive	\geq cut-off
Borderline	$0.9 \times \text{cut-off} - \text{cut-off}$
Negative	$<$ cut-off

It is recommended that each laboratory establishes its own normal and pathological reference ranges.

Performance Characteristics

Precision

To determine precision, 3 stool samples were measured multiple times. For the determination of the intra-assay coefficient of variation (CV) samples were measured in an 8-fold determination in one test run.

The determination of the inter-assay coefficient of variation was done by an 8-fold determination on 2 days in 5 different test runs.

Sample	Intra-assay-coefficient of variation		Inter-assay- coefficient of variation	
	\bar{x} OD	CV (%)	\bar{x} OD	CV (%)
1	1.386	3.0	1.321	7.7
2	0.506	3.3	0.485	6.9
3	0.332	8.5	0.345	10.8

Sensitivity and Specificity

Sensitivity and specificity of Serazym® Clostridium difficile Toxin A+B have been determined in a retrospective study with 154 stool specimens in comparison to a commercially available ELISA.

n = 154	ELISA positive	ELISA negative
Serazym® ELISA positive	103	4
Serazym® ELISA negative	2	45

Sensitivity: 98.1 %

Specificity: 91.8 %

Cross Reactivity

Stool samples positive for one of the following pathogens did not show any cross reaction with Serazym® Clostridium difficile Toxin A+B:

Aeromonas caviae; *Aeromonas hydrophila*; *Campylobacter spec.*; *enterohemorrhagic Escherichia (E.) coli* (EHEC); *Hafnia alvei*; *Pseudomonas aeruginosa*; *Salmonella enteritidis*; *Salmonella spec.*; *Salmonella typhimurium*; *Staphylococcus aureus*, enterotoxin negative; *Staphylococcus aureus*, enterotoxin positive; *Yersinia enterocolitica* O:3.

Negative stool suspensions were spiked with the following microorganisms with a bacterial count of $\geq 10^8$ colony forming units per mL in sample buffer and tested negative in the Serazym® Clostridium difficile Toxin A+B (450 nm measurement and ≥ 620 nm reference filter < cut-off):

<i>Aeromonas hydrophila</i>	(ATCC 7966)	<i>Escherichia coli</i>	(ATCC 25922)
<i>Bacillus cereus</i>	(ATCC 11778)	<i>Klebsiella pneumoniae</i>	(ATCC 13883)
<i>Bacillus subtilis</i>	(ATCC 6633)	<i>Peptostreptococcus anaerobius</i>	(ATCC 27337)
<i>Bacteroides fragilis</i>	(ATCC 25285)	<i>Proteus vulgaris</i>	(ATCC 8427)
<i>Candida albicans</i>	(ATCC 10231)	<i>Pseudomonas aeruginosa</i>	(ATCC 10145)
<i>Campylobacter coli</i>	(ATCC 33559)	<i>Salmonella enterica Serovar enteritidis</i>	(ATCC 13076)
<i>Campylobacter jejuni</i>	(ATCC 33291)	<i>Salmonella enterica Serovar typhimurium</i>	(ATCC 14028)
<i>Citrobacter freundii</i>	(ATCC 8090)	<i>Shigella flexneri</i>	(ATCC 12022)
<i>Clostridium sordellii</i>	(ATCC 9714)	<i>Shigella sonnei</i>	(ATCC 25931)
<i>Enterobacter aerogenes</i>	(ATCC 13048)	<i>Staphylococcus aureus</i>	(ATCC 25923)
<i>Enterobacter cloacae</i>	(ATCC 13047)	<i>Staphylococcus epidermidis</i>	(ATCC 12228)
<i>Enterococcus faecalis</i>	(ATCC 29212)	<i>Vibrio parahaemolyticus</i>	(ATCC 17802)

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

Application

Antigen Detection from Culture Suspensions

Colonies of *Clostridioides difficile* grown on blood or CCFA agar for 48 h can be tested directly in Serazym® Clostridium difficile Toxin A+B. Prepare a bacterial suspension according to McFarland standard 1 (OD value at 600 nm = 0.20 - 0.25 after zero compensation with Serazym® Sample diluent A): Pipette 1000 µL of sample diluent into a tube. Transfer 2 - 4 inoculating loops of a *C. difficile* colony into the sample diluent and resuspend on a vortex mixer. If necessary, read OD value at 600 nm as described above.

100 µL should be taken for ELISA testing. If selective culture media are used the detectable amount of toxins may be reduced due to inhibitory components of these media resulting in decreased OD values in the ELISA (compared to complete medium). Therefore, using selective media for toxigenic culture requires at least the preparation of a bacterial suspension according to McFarland standard 4 (OD 600 nm > 1.0 after zero compensation with Serazym® Sample diluent A). In this case the *C. difficile* colonies of at least half of a densely covered agar plate have to be used. If required, recommendations and information of the media manufacturers should be followed, who can provide information on the effect of the additives used in the corresponding media on toxin production and thus on the general suitability of a medium for toxigenic cultures.

Assay procedure with shaker

1. Allow test reagents and required number of wells to reach room temperature (RT). Shake reagents gently before use. Avoid foaming.
2. Pipette 100 µL **CONTROL +** Positive control
100 µL **CONTROL -** Negative control
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 rpm.
4. Decant, then wash each well 5x with 300 µL diluted wash buffer. Tap the plate dry on absorbent paper if necessary.
5. Add 120 µL **CONJ BIOTIN** biotin conjugate per well.
6. Cover plate and incubate for 15 min at RT on an orbital shaker with a frequency of 500 - 700 rpm.
7. Decant, then wash each well 5x with 300 µL diluted wash buffer. Tap the plate dry on absorbent paper if necessary.
8. Add 120 µL **CONJ STREPT** streptavidin-HRP conjugate per well.
9. Cover plate and incubate for 15 min at RT on an orbital shaker with a frequency of 500 - 700 rpm.
10. Decant, then wash each well 5x with 300 µL diluted wash buffer. Tap the plate dry on absorbent paper if necessary.
11. Add 120 µL **SUBSTR** substrate per well.
12. Incubate for 15 min at RT without shaking **protected from light**.
13. Add 120 µL **STOP** stop solution per well, mix gently.
14. Read OD at 450 nm measuring filter and ≥ 620 nm reference filter with a microplate reader within 30 min following reaction stop.

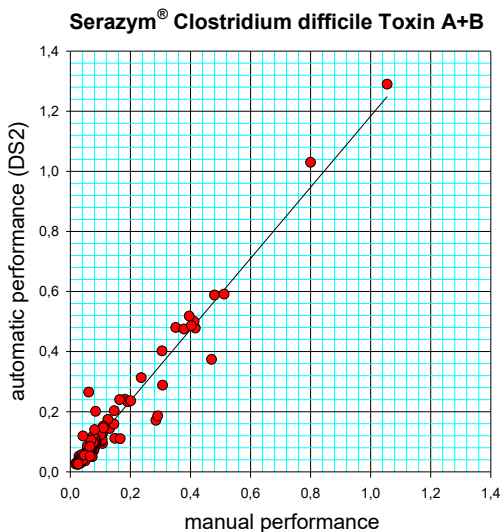
Automatic Processing

The operator is responsible for the validation of the microtiter plate processors and associated application files before using this product. Application files for the use of the automated microtiter plate processors listed below may be requested from your local distributor.

Performing Serazym® Clostridium difficile Toxin A+B on fully automated microplate processors (e.g., DS2®, DSX®, Dynex Technologies) may cause elevated absorbance values in comparison to the manual procedure caused by differences in the wash procedures and technical specifications of the equipment. In these cases, a maximum value of OD = 0.3 is permissible for the negative control. It is recommended to program a wash protocol with at least 10 s soak time per strip and wash step. A final wash step with deionized water and a soak time of 10 s is recommended after each wash cycle. If necessary, the number of washing steps may be increased to 7x or 8x.

Correlation: manual – automatic processing

A panel of 125 stool specimens was processed manually and automatically in parallel (DS2®, Dynex Technologies). The correlation coefficient was calculated at $r = 0.976$.



Change History

Version	Section	Modifications
2026-04	Cover sheet	Adjustment of REF number to packaging concept
	Test Components (Delivery Scope)	Adjustment of volumes to packaging concept, addition of quantity or concentration of the active ingredient
	Additional Materials and Aids Required for the Test Procedure	Addition of "reagent container for multi-channel micropipettes"
	Important information	Addition of negative control as a component across lots and products; Table under "Safety instructions":
	Sample Treatment	Adjustment to the labeling on the label
	Assay Procedure	Addition of sample vessel example
		<u>Adaptation to packaging concept</u>
2026-05	Application: Automatic Processing	Addition of user responsibility for the validation of microtiter plate processors