Instructions for Use

Serazym® Clostridium difficile GDH

Enzyme immunoassay for the qualitative detection of *C. difficile* specific glutamate dehydrogenase in stool samples of human origin

REF	E-107	Σ	96
IVD	In-vitro-diagnostic medical device		CE

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

Intended Use

Serazym® Clostridium difficile GDH is an IVD test for the qualitative determination of *Clostridioides (formerly: Clostridium) difficile*-specific glutamate dehydrogenase (GDH) in human stool samples through manual or semi-automatic processing by a laboratory professional user.

It is intended for screening and as an aid in the diagnosis of *C. difficile* infection (CDI) in samples from patients presenting with symptoms of *C. difficile*-associated diarrheal disease. In GDH-positive samples, the test can be combined with subsequent detection of toxins A and B to rule out toxigenic strains (two-step diagnostic approach).

Principle of the Test

Serazym® Clostridium difficile GDH is an enzyme immunoassay based on poly- and monoclonal antibodies against *Clostridioides difficile* glutamate dehydrogenase (GDH). Diluted, untreated stool samples as well as negative and positive controls are dispensed simultaneously with peroxidase (HRP)-labeled monoclonal anti-*C. difficile* GDH antibodies into the wells of the microtiter plate coated with polyclonal anti-*C. difficile* GDH antibodies. After incubation unbound components are removed by a washing step, then 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 GDH antigen.

Test Components (Delivery Scope)

			For 96 wells
1	**WELLS**	**Microtiter plate** coated with < 5 µg/mL polyclonal anti-*C. difficile* GDH antibodies (sheep)	12 single breakable 8-well strips, green 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** < 1 µg/mL recombinant *C. difficile* 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	**CONJ HRP**	**HRP conjugate** < 5 µg/mL HRP-labeled, monoclonal anti-*C. difficile* GDH antibodies (mouse)	15 mL, ready to use, colored green, 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 pipette or multi-pipette with pipette tips • reagent reservoirs for multi-channel pipetting • 8-channel wash comb with vacuum pump and waste bottle or microtiter plate washer • microtiter plate reader with 450 nm measuring filter and ≥ 620 nm reference filter • deionized water • measuring cylinder • tubes for sample preparation

Important Information

This device is for *in-vitro* diagnostic use only. Follow the instructions carefully. The kit may be used by laboratory 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 wash buffer (10x), sample diluent, negative control, substrate and stop solution.
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 GDH 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. Positive and negative controls are ready to use.

For larger sample series, pipetting reagents from liquid reservoirs using a multichannel pipette 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 Material 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)	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. EUH210: Safety data sheet available on request. 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
DIL	Contains material of animal origin. Preservative: < 0.1 % sodium azide
CONTROL +	Contains material of microbial and animal origin. Preservative: < 0.1 % sodium azide
CONTROL –	Contains material of animal origin. Preservative: < 0.01 % sodium azide
CONJ HRP	EUH210: Safety data sheet available on request. Contains material of animal origin. Preservative: < 0.01 % 5-bromo-5-nitro-1,3-dioxane
SUBSTR	Hazard component: 2-pyrrolidone Signal word: Danger  H360: May damage fertility or the unborn child. P201: Obtain special instructions before use. P280: Wear protective gloves/protective clothing/eye protection/face protection. P308+P313: IF exposed or concerned: Get medical advice/attention. Restricted to professional users. Preservatives: < 0.00015 % reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)
STOP	-

Limitations of the Procedure
Qualitative enzyme immunological detection of *Clostridoides difficile* GDH in stool samples is not correlated with the diagnosis of *C. difficile*-associated disease. *C. difficile*-associated diseases are caused by toxins A and B of pathogenic *C. difficile* strains. Therefore, if testing for GDH is positive, subsequent testing for toxins A and B must be performed to determine if the strain is toxigenic. Conversely, a negative result in Serazym® Clostridium difficile GDH does not exclude an infection. False negative tests may result from improper timing of sample collection or inhomogeneous antigen distribution in the sample. The qualitative enzyme immunological detection of *Clostridoides difficile* GDH in stool samples does not allow correlation between the measured OD and the severity of infection. The OD of the specimen may not correlate with the OD of the positive control. Cross contamination of reagents and samples may result in false positive results. Incorrect dilutions, insufficiently homogenized samples, and particles not sedimented by centrifugation may cause false negative as well as false positive test results. 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.
Pipette 500 µL sample buffer into a reaction tube. For solid or semi-solid stool samples transfer 100 mg (diameter approx. 2 - 3 mm) 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 refrigerated with desiccant in the original bag carefully resealed. 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 µL **CONJ HRP** HRP conjugate per well.
3. Pipette 100 µL **CONTROL +** Positive control
   100 µL **CONTROL –** Negative control
   100 µL diluted stool specimen each, mix gently.
4. Cover the plate and incubate for 60 min at RT.
5. Decant, then wash each well 5x with 300 µL diluted wash buffer.
   Tap the plate dry on absorbent paper if necessary.
6. Add 100 µL **SUBSTR** substrate per cavity.
7. Incubate for 10 min at RT **protected from light**.
8. Add 100 µL **STOP** stop solution per well, mix gently.
9. Read OD at 450 nm measuring and ≥ 620 nm reference filter with a microtiter plate reader within 30 min following reaction stop.

Evaluation of Results

Qualitative Evaluation:
Cut-off determination: OD negative control + 0.10
Samples showing OD values equal with or higher than the cut-off are considered positive, samples with OD values below cut-off are considered negative.

The test run is valid if

- the mean OD value of the negative control is ≤ 0.20 (manual processing)
  ≤ 0.30 (automatic processing)
- the mean OD value of the positive control is ≥ 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	≥ 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, 4 stool samples were measured multiple times. For the determination of the intra-assay coefficient of variation samples were measured in an 8-fold determination in one test run. The determination of the inter-assay coefficient of variation was done by double determination in 5 different test runs. The lot-to-lot coefficient of variation was determined by single determination in 3 lots of the product.

Sample	Intra-assay coefficient of variation		Inter-assay coefficient of variation		Lot-to-Lot coefficient of variation	
	$\bar{x}$ OD	CF (%)	$\bar{x}$ OD	CF (%)	$\bar{x}$ OD	CF (%)
1	2.800	1.52	1.707	14.6	1.840	9.0
2	1.960	2.46	1.155	12.8	1.285	13.4
3	0.611	7.19	0.895	10.9	1.071	11.4
4	0.352	5.15	0.349	26.6	0.026	17.6

Detection Limit
The lower detection limit of glutamate dehydrogenase (GDH) in Serazym® Clostridium difficile GDH was determined at 10 ng/mL by titration of recombinant GDH antigen.

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

Sensitivity and specificity of the Serazym® ELISA have been determined in two retrospective studies with 235 and 170 stool specimens, respectively, in comparison to a commercially available ELISA.

Study 1

n = 235	**ELISA 1 positive**	**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 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	**ELISA 2 positive**	**ELISA 2 negative**
Serazym® ELISA positive	69	1*
Serazym® ELISA negative	3	97

Sensitivity: 95.8 %
Specificity: 99.0 %
** This sample was confirmed positive by PCR. Specificity amended: 100 %

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

Aeromonas hydrophila	(ATCC 7966)	*Klebsiella pneumoniae*	(ATCC 13883)
Bacillus cereus	(ATCC 11778)	*Peptostreptococcus anaerobius*	(ATCC 27337)
Bacillus subtilis	(ATCC 6633)	*Proteus vulgaris*	(ATCC 8427)
Bacteroides fragilis	(ATCC 25285)	*Pseudomonas aeruginosa*	(ATCC 10145)
Candida albicans	(ATCC 10231)	*Salmonella enterica serovar enteritidis*	(ATCC 13076)
Campylobacter coli	(ATCC 33559)	*Salmonella enterica serovar typhimurium*	(ATCC 14028)
Campylobacter jejuni	(ATCC 33291)	*Shigella flexneri*	(ATCC 12022)
Citrobacter freundii	(ATCC 8090)	*Shigella sonnei*	(ATCC 25931)
Clostridium sordellii	(ATCC 9714)	*Staphylococcus aureus*	(ATCC 25923)
Enterobacter aerogenes	(ATCC 13048)	*Staphylococcus epidermidis*	(ATCC 12228)
Enterobacter cloacae	(ATCC 13047)	*Vibrio parahaemolyticus*	(ATCC 17802)
Enterococcus faecalis	(ATCC 29212)	*Vibrio cholerae*	Clinical isolate
Escherichia coli	(ATCC 25922)	*Yersinia enterocolitica serotypes O3, O9*	Clinical isolate

Interference
None of the following substances in the indicated concentrations added to *Clostridioides difficile* GDH positive and negative stool samples did show a significant impact on the test result:
Barium sulfate (5 %), Buscopan® (2 mg/mL), Cyclamate (5 %), Diclofenac (2 mg/mL), human hemoglobin (5 mg/mL), Hylak® N (5 %), Iberogast® (5 %), Imodium® akut duo (0.2 / 12.5 mg/mL), 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 %).

Application

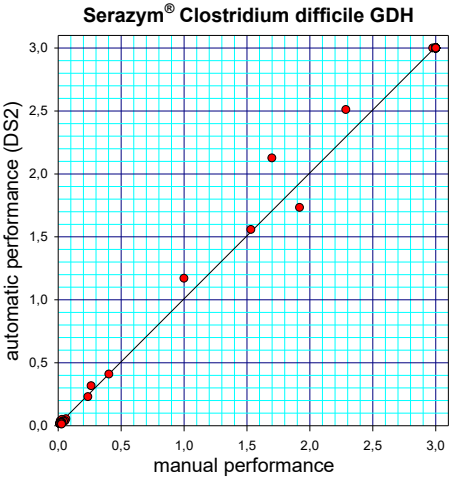
Working steps with shaker

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 **CONJ HRP** HRP conjugate per well.
3. Pipette 100 µL **CONTROL +** Positive control
   100 µL **CONTROL –** Negative control
   100 µL diluted stool specimen each, mix gently.
4. Cover the plate and incubate for 30 min at RT on an orbital shaker with a frequency of 500 – 700 rpm.
5. Decant, then wash each well 5x with 300 µL diluted wash buffer.
   Tap the plate dry on absorbent paper if necessary.
6. 100 µL **SUBSTR** substrate per cavity.
7. Incubate for 10 min at RT **protected from light**.
8. 100 µL **STOP** stop solution per well, mix gently.
9. Read OD at 450 nm measuring and ≥ 620 nm reference filter with a microtiter plate 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 GDH on automated microtiter plate 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 90 stool specimens was processed manually and automatically in parallel (DS2®, Dynex Technologies). The correlation coefficient was calculated at r = 0.999.



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; erledigtTable under “Safety instructions”: Adjustment to the labeling on the label
	Sample Treatment	Addition of sample vessel example
	Assay Procedure	Adaptation to packaging concept
2026-05	Application: Automatic Processing	Addition of user responsibility for the validation of microtiter plate processors