












Serazym[®] H. pylori 2nd Gen.

Enzyme immunoassay for the qualitative detection of *Helicobacter pylori*-specific antigen in stool samples of human origin

REF	E-114	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
Σ Sufficient for <i>n</i> tests	 Biohazard	 Use-by date
		 Attention

Intended Use

Serazym® *H. pylori* 2nd Gen. is an IVD test for the qualitative determination of *Helicobacter pylori*-specific antigen in stool samples of human origin through manual or semi-automatic processing by a laboratory professional user.

It is intended to aid in the diagnosis of a *H. pylori* infection in samples from patients with symptoms of a *H. pylori*-associated gastroenteritis, for the monitoring of an infection during eradication therapy and after eradication therapy.

Principle of the Test

Serazym® *H. pylori* 2nd Gen. is an enzyme immunoassay assay based on monoclonal antibodies against *Helicobacter pylori* antigen. Diluted, untreated stool samples as well as negative and positive control samples are dispensed into wells of the microtiter plate coated with monoclonal anti-*Helicobacter pylori* antibodies. After incubation, unbound components are removed by a washing step and peroxidase (HRP)-labeled monoclonal anti-*Helicobacter pylori* antibodies are dispensed into the wells. 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. After incubation 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 *Helicobacter pylori* antigens.

Test Components (Delivery Scope)

		For 96 wells
1	WELLS	Microtiter plate coated with < 5 µg/mL monoclonal anti- <i>H. pylori</i> antibodies (mouse)
		12 single breakable 8-well strips, wine-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 < 1.5 µg/mL native <i>H. pylori</i> -reactive sample (inactivated)
		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 < 10 µg/mL HRP-labeled monoclonal anti- <i>H. pylori</i> 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

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 microtiter plate washer • microplate 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. Imprinted expiry dates 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[®] H. pylori 2nd Gen. 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 only be used once. Each sample and control should 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)	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); < 0.01 % 5-bromo-5-nitro-1,3-dioxane
DIL	Contains material of animal origin. Preservatives: < 0.1 % sodium azide
CONTROL +	Contains material of microbial and animal origin. Preservatives: < 0.1 % sodium azide
CONTROL -	Contains material of animal origin. Preservatives: < 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

The qualitative enzyme immunological detection of *Helicobacter pylori* antigen in stool samples does not allow 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 positive results. Incorrect dilutions, insufficiently homogenized samples, and particles not sedimented by centrifugation may cause false positive as well as false negative test results. A negative test result obtained with Serazym® H. pylori 2nd Gen. does not exclude an *H. pylori* 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 repeated testing 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 refrigerated with desiccant in the original bag carefully resealed. Dilute wash buffer (10x) 1 : 10 with deionized water.

Example: 10 mL wash buffer (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 each.
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 100 μ L **CONJ HRP** HRP conjugate per well.
6. Cover plate and incubate for 30 min at RT.
7. Decant, then wash each well 5x with 300 μ L diluted wash buffer. Tap the plate dry on absorbent paper if necessary.
8. Add 100 μ L **SUBSTR** Substrate per well.
9. Incubate for 10 min at RT **protected from light**.
10. Add 100 μ L **STOP** Stop solution per well, mix gently.
11. Read OD at 450 nm measuring filter and ≥ 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 + 0.10

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 for *Helicobacter pylori*-specific antigen.

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 ≥ 1.20

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
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 a 24-fold determination in one test run.

The determination of the inter-assay coefficient of variation was done by a 3-fold determination on 2 days in 10 different test runs. The lot-to-lot coefficient of variation was determined by 3-fold 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	CV (%)	\bar{x} OD	CV (%)	\bar{x} OD	CV (%)
1	2.377	5.3	2.483	6.3	2.626	10.4
2	0.871	7.4	1.044	13.2	1.265	17.4
3	0.124	11.0	0.142	10.3	0.162	18.5

Detection Limit

The lower limit of detection of Serazym® *H. pylori* 2nd Gen. has been determined by titration of negative stool samples spiked with purified *H. pylori* lysate antigen. The lower detection limit is < 31.3 ng/mL.

Determination of the cut-off value

Based on ROC analysis of 333 natural stool samples the cut-off value in Serazym® *H. pylori* 2nd Gen. has been determined with an absorbance for the negative control of + 0.1.

Sensitivity and Specificity

Sensitivity and specificity of Serazym® *H. pylori* 2nd Gen. have been determined in a retrospective study in comparison to two commercially available ELISA.

n = 233	ELISA 1 positive	ELISA 1 negative
Serazym® ELISA positive	140	6**
Serazym® ELISA negative	2*	85

Sensitivity: 98.6 %

Specificity: 93.4 %

Samples labeled with * and ** were re-tested in a commercially available lateral flow assay. This results in a corrected sensitivity of 99.3 % and a corrected specificity of 97.7 %. Compared to ELISA 1 the accuracy is 98.7 %.

n = 79	ELISA 2 positive	ELISA 2 negative
Serazym® ELISA positive	53	5***
Serazym® ELISA negative	0	21

Sensitivity: 100 %

Specificity: 80.8 %

Samples labeled with *** were confirmed positive by comparative ELISA 1. This results in a corrected specificity of 100 %. Compared to ELISA 2 the accuracy is 100 %.

Cross Reactivity

Negative stool suspensions were spiked with the following microorganisms with a bacterial count of $\geq 10^8$ colony-forming units (cfu) per mL in sample buffer and tested negative in the Serazym® H. pylori 2nd Gen (450 nm measuring filter and ≥ 620 nm reference filter < cut-off):

<i>Aeromonas hydrophila</i>	ATCC 7966	<i>Campylobacter coli</i>	ATCC 33559
<i>Bacillus cereus</i>	ATCC 11778	<i>Campylobacter jejunii</i>	ATCC 32291
<i>Bacillus subtilis</i>	ATCC 6633	<i>Campylobacter fetus</i>	ATCC 27374
<i>Bacteroides fragilis</i>	ATCC 25285	<i>Campylobacter upsaliensis</i>	ATCC 43954
<i>Citrobacter freundii</i>	ATCC 8090	<i>Campylobacter lari</i>	ATCC 35221
<i>Clostridium sordelli</i>	ATCC 9714	<i>Vibrio cholerae</i>	clinical isolate
<i>Enterobacter aerogenes</i>	ATCC 13048	<i>Yersinia enterocolitica</i> 0:3	clinical isolate
<i>Enterobacter cloacae</i>	ATCC 13047	<i>Yersinia enterocolitica</i> 0:9	clinical isolate
<i>Enterococcus faecalis</i>	ATCC 29212	<i>Yersinia enterocolitica</i> Y11	clinical isolate
<i>Escherichia coli</i>	ATCC 25922	<i>Yersinia enterocolitica</i> RKI 0803733	clinical isolate
<i>Klebsiella pneumoniae</i>	ATCC 13883	<i>Clostridium difficile</i>	VPI 10463
<i>Peptostreptococcus anaerobius</i>	ATCC 27337	<i>Salmonella infantis</i>	ATCC 51741
<i>Proteus vulgaris</i>	ATCC 8427	<i>Salmonella anatum</i>	ATCC 9270
<i>Pseudomonas aeruginosa</i>	ATCC 10145	<i>Salmonella paratyphi</i> A	ATCC 11511
<i>Salmonella enterica</i> serovar <i>thyphimurium</i>	ATCC 14028	<i>Salmonella paratyphi</i> B	ATCC 8759
<i>Salmonella enterica</i> ssp. <i>galolyticus/enteritidis</i>	ATCC 13076	<i>Salmonella paratyphi</i> C	Nr. 2 Pasteur
<i>Shigella flexneri</i>	ATCC 12022	<i>Lactococcus lactis</i>	DSM 20481
<i>Shigella sonnei</i>	ATCC 25931	<i>Proteus mirabilis</i>	ATCC 29906
<i>Staphylococcus aureus</i>	ATCC 25923	<i>Pseudomonas fluorescens</i>	ATCC 13525
<i>Staphylococcus epidermidis</i>	ATCC 12228	<i>Pseudomonas putida</i>	ATCC 49128
<i>Vibrio parahaemolyticus</i>	ATCC 17802	<i>Streptococcus agalactiae</i>	ATCC 13813
<i>Candida albicans</i>	ATCC 10231	<i>Morganella morganii</i>	ATCC 25830

Interference

None of the following substances in the indicated concentrations added to *Helicobacter pylori* positive and negative stool samples did show a significant impact on the test result:

(-)-Scopolamine N-butyl bromide (0.5 %, Buscopan®), barium sulfate (5 %), bismuth(III) subsalicylate (0.5 %, Pepto-Bismol), Cyclamat (5 %), Diclofenac (0.5 %), hemoglobin human (5 %), blood human (5 %), Hylak® N (5 %), Iberogast® (5 %), Imodium® akut duo (0.03/1.9 %), Loperamid hydrochloride (5 %, Loperamid-CT akut), Metronidazole (0.5 %), Mucin (5 %), Nexium® (0.03 %), Nifuroxazide (0.5 %, Pentofuryl®), palmitic acid (20 %), Perenterol forte (0.5 %), Rennie® (20 %), Simagel® (1 %), stearic acid (20 %), Vancomycin (0.5 %).

Clinical Performance

Seven clinical studies have been carried out with comparative ELISA 1 in Europe and the Arabian region between 2001 and 2005, which demonstrated the clinical performance of comparative ELISA 1. The comparative tests with Serazym® H. pylori 2nd Gen. result in the following data:

Diagnostic sensitivity	99.3 %
Diagnostic specificity	97.7 %
Positive predictive value	98.6 %
Negative predictive value	98.9 %
Likelihood ratio	43.2 (LR+) 0.007 (LR-)

Four clinical studies have been carried out with comparative ELISA 2 in Europe and Asia between 2008 and 2020, which demonstrated the clinical performance of comparative ELISA 2. The comparative tests with Serazym® H. pylori 2nd Gen. result in the following data:

Diagnostic sensitivity	100 %
Diagnostic specificity	100 %
Positive predictive value	100 %
Negative predictive value	100 %
Likelihood ratio	LR+ → +∞ LR- → 0

Application

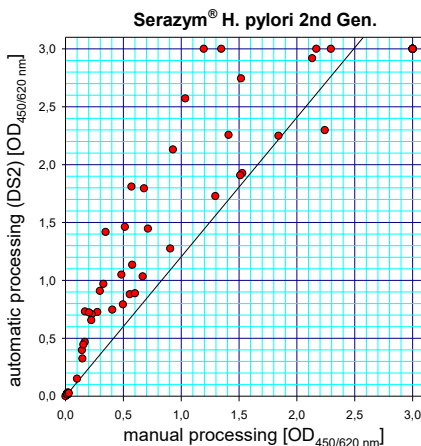
Automatic Processing

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.

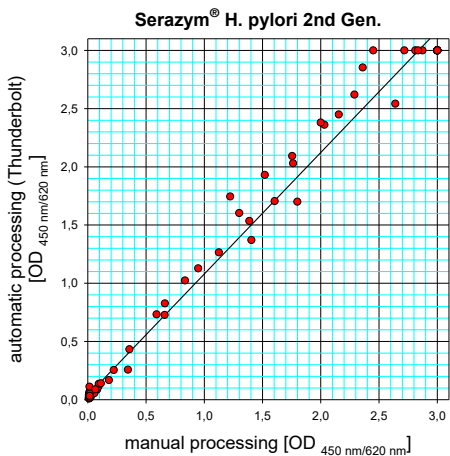
Performing Serazym® H. pylori 2nd Gen. on fully automated microplate processors (e.g., DS2®, DSX®, Dynex Technologies or ThunderBolt®, Gold Standard Diagnostics) may cause elevated absorbance values in comparison to the manual procedure caused by differences in the wash procedures and technical specifications 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 wash steps may be increased to 7x or 8x.

Correlation: manual – automatic processing

A panel of 93 stool specimens was processed manually and automatically in parallel (DS2®, Dynex Technologies). The correlation coefficient was calculated at $r = 0.949$.



A panel of 94 stool specimens was processed manually and automatically in parallel (ThunderBolt®, Gold Standard Diagnostics). The correlation coefficient was calculated at $r = 0.995$.



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": Adjustment to the labeling on the label
	Sample Treatment	Addition of sample vessel example
	Assay Procedure	Adaptation to packaging concept
	Application	Updating DS2 Data in the Application
2026-05	Application: Automatic Processing	Addition of user responsibility for the validation of microtiter plate processors