Serazym® Giardia

Enzyme immunoassay for detection of *Giardia lamblia* CWP-1 in faecal samples

REF E-106-A 96 IVD *In-vitro*-diagnostic medical device CE

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Introduction

Giardia lamblia (syn. *Lamblia intestinalis*) is one of the most common intestinal protozoan pathogens worldwide. The incidence strongly depends on the geographic region and reaches 2 - 7% in central Europe and exceeds 50% in tropical countries (1, 2).
The clinical picture of a *Giardia lamblia* infection ranges from the asymptomatic carrier state to acute diarrhea often accompanied by abdominal pain and flatulence. Chronic giardiasis may cause severe malabsorption syndrome. After the incubation time of about 5 days symptoms last for about 5 days in acute cases and up to 2 months in chronic courses of the disease.
The life cycle of *Giardia lamblia* is characterized by two stages: the trophozoite and the cyst stage. The trophozoite is the motile dividing stage and inhabits the upper small intestine. Ascending infections of the gallbladder may also occur. The cyst is the infective form of the parasite. It develops in the intestine and is excreted with the faeces. Cysts are transmitted via contaminated food or drinking water but also from person to person. The ability of infecting humans as well as different mammalian species explains the zoonotic potential of this parasite (1, 2).
External factors (pH-value, composition of bile salts) induce the differentiation from trophozoites to cysts (3, 4, 5). During the process of encystation which takes about 12 to 48 hours proteins and glycopolymeric substances needed for the cyst wall formation are produced by the trophozoites. These substances are stored in encystation specific vesicles (ESV) within the cytoplasma of the trophozoites, transported to the cell surface, excreted by exocytosis and incorporated into the cyst wall.

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The **c**yst **w**all **p**roteins CWP-1 and CWP-2 as the main components of the cyst wall have a molecular weight of 26 and 29 kDa resp. (2, 4, 7), and they form heterodimers of 65 kDa which are also known as **G**iardia **S**pecific **A**ntigen, GSA-65 (6). Encystation is a continuous and often incomplete process leading to the release of cyst wall proteins into the intestine during the progeny of the parasite (2).
The immunological detection of Giardia specific cyst wall proteins by enzyme immunoassay for the diagnosis of acute Giardia infections is established (10). In contrast to microscopy the detection of soluble parasite antigens has the advantage that morphologically intact cysts or trophozoites are not essentially needed (8, 9).

References:

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Intended use

***Serazym*® Giardia is an *in-vitro*-diagnostic medical device for direct detection of *Giardia lamblia* cyst wall protein 1 (CWP-1) in faecal samples.**

Principle of the test

The *Serazym*® Giardia is based on polyclonal antibodies to *Giardia lamblia* cyst wall protein 1 (CWP-1). Within the first incubation step (60 min, room temperature) diluted stool specimens as well as positive and negative controls react with the solid-phase adsorbed polyclonal antibodies. Unbound components are removed by a subsequent washing step. Within the second incubation step (30 min, room temperature) solid-phase bound immune complexes react with the horseradish (HRP)-labelled polyclonal antibodies of the conjugate. Unbound reagents are separated from the solid-phase antibody-antigen-antibody immune complexes by a further washing step. HRP converts the subsequently added colourless chromogenic substrate solution into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells after 10 min incubation at room temperature 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 *Giardia lamblia* cyst wall protein. Referring to the cut-off value results are interpreted as positive or negative.

Test components

			For 96 Wells
1	WELLS	**Microtitration plate** coated with polyclonal anti-Giardia CWP-1 antibodies (sheep)	12 single breakable 8 well strips colour coding white vacuum-sealed with desiccant
2	WASHBUF CONC 10x	**Wash buffer** 10-fold	100 ml concentrate for 1000 ml solution white cap
3	DIL	**Sample diluent**	100 ml · ready to use coloured yellow black cap
4	CONTROL +	**Positive control** Recombinant *Giardia lamblia* Cyst wall proteins	2.0 ml · ready to use coloured blue red cap
5	CONTROL –	**Negative control** *Giardia lamblia* negative sample	2.0 ml · ready to use coloured blue green cap
6	CONJ HRP	**HRP-conjugate** HRP-labelled polyclonal anti-Giardia CWP-1 antibodies (sheep)	15 ml · ready to use coloured green violet cap
7	SUBSTR TMB	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml · ready to use blue cap
8	STOP	**Stop solution** 0.25 M sulphuric acid	15 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 -20°C. Repeated freezing and thawing of samples should be avoided. Stool samples already diluted with the *Serazym*® sample diluent can be stored over 72 h at 2…8°C before testing. Faecal samples that are already diluted in transportation media (PARA-PAK PLUS, PARA-PAK PLUS SAF) may cause decreased OD values in the *Serazym*® Giardia in comparison to untreated samples. In case of samples with low antigen concentration OD values may decrease beyond the detection limit of the test and therefore it is recommended to use untreated samples. If untreated samples are not available the already prediluted sample should be further diluted with *Serazym*® sample diluent as stated below.

Preparation

Fresh, untreated samples

Quickly thaw frozen samples. Warm samples to room temperature and mix well. Pipette 1000 µl of sample diluent into a clean tube. Using a disposable stirring rod transfer about 200 mg (diameter about 4 - 6 mm) of faeces if solid or 200 µl if liquid into the tube and suspend thoroughly. If necessary, sediment floating particles by a centrifugation step with a micro centrifuge for one min at maximum speed.

Conserved samples, prediluted in transportation media

Mix sample thoroughly. Pipette 250 µl sample diluent into a clean tube, transfer 1000 µl of the prediluted sample to this tube and mix well.

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 · microplate reader with optical filters of 450 nm for measurement and ≥ 620 nm for reference · distilled or deionized water · glassware · tubes (2 ml) for sample preparation

Preparation and storage of reagents

Kit size and expiry

One kit is designed for 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 two 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.
Example: 10 ml wash buffer concentrate *(2)* + 90 ml distilled or deionized water.

Assay procedure

Dilute samples with sample diluent *(3)* 1 : 6, e.g. 200 mg or 200 µl faeces + 1.0 ml sample diluent *(3)*.
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 light exposure of the TMB substrate solution!

Working steps

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 sample**.
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. 3 drops (or 100 µl) **CONJ HRP** HRP-conjugate *(6)* 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. 3 drops (or 100 µl) **SUBSTR TMB** substrate *(7)* per well.
9. Incubate for 10 min at RT protected from light.
10. 3 drops (or 100 µl) **STOP** stop solution *(8)* per well, mix gently.
11. 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 with or higher than the cut-off are considered positive,
samples with OD values below the cut-off are considered negative for *Giardia lamblia* antigen.

Reference values

Serazym® Giardia	
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:

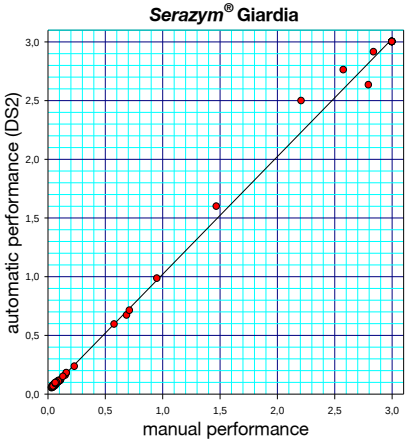
- the mean OD of the negative control is ≤ 0.20 (manual performance)
  ≤ 0.30 (automatic performance)
- the mean OD of the positive control is: ≥ 0.80

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 the absorbance of a sample 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 diluted sample can cause false negative as well as false positive results.
A negative test result not necessarily excludes a *Giardia lamblia* infection. Inhomogeneous antigen distribution in the sample can cause false negative results. Therefore in case of a negative test result but clinical suspicion of an infection a further sample of the respective person should be investigated.
A final interpretation of the test result should always consider clinical findings as well.

Automatic processing
Performing the *Serazym*® Giardia 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 90 faecal specimens was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with 0.999.



Performance characteristics

Precision
Intra-Assay coefficient of variation (CV) in the *Serazym*® Giardia from 8-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	2.182	0.098	4.79
2	1.175	0.043	3.92
3	0.563	0.027	5.08
4	0.179	0.006	3.59

Inter-Assay coefficient of variation (CV) in the *Serazym*® Giardia in 8 different test runs from twofold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	1.947	0.144	7.42
2	1.732	0.149	8.63
3	0.760	0.058	7.69
4	0.217	0.020	9.04

Lower detection limit
The lower detection limit of Giardia antigen in the *Serazym*® Giardia was determined by titration of cysts, trophozoites and recombinant CWP-1, diluted in a negative stool sample:
1.6 x 10^3 cysts, 6.3 x 10^4 trophozoites and 3.1 ng CWP-1 per ml stool suspension.

Specificity and sensitivity
Specificity: In all n = 320 stool samples from routine microbiology laboratory were investigated by the *Serazym*® Giardia: Negative: n = 317**,** Positive: n = 3**,** Specificity: 99.1%.
The 3 ELISA positive samples were reinvestigated by direct immunofluorescence and confirmed as true positive. Specificity amended: 100%.
Sensitivity: Human stool samples designated Giardia positive were tested in the *Serazym*® Giardia in comparison to 2 commercially available ELISAs.

n = 33 Human stool samples	ELISA 1 positive	ELISA 1 negative
Serazym® ELISA positive	30	0
Serazym® ELISA negative	1	2

Sensitivity compared to ELISA 1: 96.8%

n = 23 Human stool samples	ELISA 2 positive	ELISA 2 negative
Serazym® ELISA positive	19	2*
Serazym® ELISA negative	0	2

Sensitivity compared to ELISA 2: 100%

* Both samples with a positive test result in the *Serazym*® Giardia and a negative test result in ELISA 2 were confirmed true positive by direct immunofluorescence and ELISA 1.
Faecal samples from animals belonging to 7 different species were tested by *Serazym*® Giardia in comparison to 2 commercially available assays.

n = 60 animal faecal samples	ELISA 1 positive	ELISA 1 negative
***Serazym*® ELISA positive**	48	0
***Serazym*® ELISA negative**	4	8

Sensitivity compared to ELISA 1: 92.3%

n = 54 animal faecal samples	ELISA 2 positive	ELISA 2 negative
***Serazym*® ELISA positive**	40	7*
***Serazym*® ELISA negative**	1**	6

Sensitivity compared to ELISA 2: 97.6%

* The 7 samples with negative test result in ELISA 2 but positive in the *Serazym*® ELISA were confirmed true positive by direct immunofluorescence and ELISA 1.
** The *Serazym*® ELISA negative but ELISA 2 positive sample was tested negative by direct immuno-fluorescence and ELISA 1.

In all 88 samples (human and animal samples) designated Giardia positive were tested in parallel by *Serazym*® Giardia and direct immuno fluorescence.

n = 88 faecal and stool samples	IFT positive	IFT negative
***Serazym*® ELISA positive**	78	1*
***Serazym*® ELISA negative**	1	8

Sensitivity compared to IFT: 98.7%

* The ELISA positive, IFT negative sample reacted positive in both comparative ELISAs 1 and 2 as well.

Cross reactivity
Stool samples positive for one of the subsequent pathogens have been tested with the *Serazym*® Giardia and showed no cross reactivity:
Adenovirus (n = 2), *Rotavirus* (n = 6), *Astrovirus* (n = 4), *Norovirus* (n = 6), *Clostridium difficile* (n = 2), *Campylobacter jejuni* (n = 6), *Helicobacter pylori* (n = 7), *Salmonella ssp.* (n = 9), *Ancylostoma duodenale* (n = 1), *Ascaris lumbricoides* (n = 2), *Blastocystis hominis* (n = 1), *Cryptosporidium parvum* (n = 4), *Entamoeba dispar/histolytica* (n = 7).

Negative stool samples have been spiked with $\geq 10^8$ colony forming units per ml of the following microorganisms and were tested negative with the *Serazym*® Giardia (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 positive and negative stool samples showed a significant impact on the test result:
Barium sulfate (5%), Buscopan® (2 mg/ml), Cyclamate (5%), Diclofenac (2 mg/ml), Hemoglobine human (5 mg/ml), Blood human (5%), Hylak® N (5%), Iberogast® (5%), Immodium® 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 (2 mg/ml).

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
2020-10-19	Intended use	Correction
	Assay procedure	Update
	Cross reactivity	Extension
	Common Advices and Precautions	Update
	History of Changes	New section “History of Changes“

Incubation Scheme *Serazym*® Giardia (E-106)

1.		pipette	
		100 µl	CONTROL + *(4)*
		100 µl	CONTROL – *(5)*
		100 µl	**diluted stool sample**
		60 min	incubation (room temperature)
		5 x wash	with wash solution
2.		3 drops (or 100 µl)	CONJ HRP *(6)*
		30 min	incubation (room temperature)
		5 x wash	with wash solution
3.		3 drops (or 100 µl)	SUBSTR TMB *(7)*
		10 min	incubation (room temperature) protected from light
4.		3 drops (or 100 µl)	STOP *(8)*

Read OD at 450 / ≥ 620 nm