RIDASCREEN® Entamoeba

REF C1701

R-Biopharm AG, An der neuen Bergstraße 17, 64297 Darmstadt, Germany
Phone: +49 (0) 61 51 81 02-0 / Fax: +49 (0) 61 51 81 02-20

1. Intended use

For *in vitro* diagnostic use. RIDASCREEN® Entamoeba is an enzyme immunoassay for the qualitative identification of *Entamoeba histolytica* and *Entamoeba dispar* in human stool samples**.**

2. Summary and explanation of the test

Every year, up to 500 million people become infected with *Entamoeba histolytica and Entamoeba dispar* worldwide. Molecular genetic analyses have shown that these protozoa, which were identified by traditional diagnostic methods and designated as *Entamoeba histolytica*, are two species with a morphology that cannot be differentiated. One is the pathogenic species *Entamoeba histolytica* and the other is *Entamoeba dispar*, which is not pathogenic in the current state of knowledge.
The clinical symptoms of amoebiasis are triggered by invasion of the parasite from the intestinal lumen into the mucous membrane of the colon. Here one often finds trophozoites with phagocyted erythrocytes. Due to their size, these trophozoites are known as the magna form. The results of invasion into the intestinal mucosa are diarrhea, dysentery, or even amoeboma. Upon dissemination, the potential complications include abscesses in the liver, the lungs, and in very rare cases even in the brain, most often with a fatal course of disease if not treated.
The clinical symptoms of the acute intestinal form of amoebiasis are painful abdominal cramps with nausea and massive diarrhea with blood and mucous in the feces. The acute stage of disease can develop into a chronic condition with occasional diarrhea that alternates with constipation, abdominal pain, nausea, and vomiting. The literature includes descriptions of completely asymptomatic carriers of the cysts.
For direct identification of the pathogens, sensitive immunological test methods such as RIDASCREEN® ELISA with adhesin-specific antibodies are much more advantageous than microscopy. The specific adhesin of Entamoeba is a surface protein which binds specifically to the galactose or N-acetyl-galactosamine of the enterocytes of the host and facilitates sub-sequent invasion by the pathogen by lysis of these cells.
About 10 % of all cases of acute amoebic dysentery are followed by extraintestinal complications such as abscesses in the liver or the infection of other organs. Identification of antibodies by serology is indicated in the case of extraintestinal amoebiasis.
This method makes the diagnosis independent of subjective assessment and it is more sensitive in detecting the parts that are no longer morphologically identifiable. The antibody titers can usually be determined at the same time when clinical signs and symptoms begin, and the immediately following identification of specific antibodies makes it possible to identify *E. histolytica*. This also offers the possibility of differentiation between the titer levels of intestinal and extraintestinal amoebiasis, which is important in the choice of treatments.

3. Test principle

The RIDASCREEN® Entamoeba Test uses specific antibodies in a sandwich-type method. The well surface of the microwell plate is coated with specific antibodies to the antigens of *Entamoeba histolytica* and *Entamoeba dispar.* A pipette is used to place a suspension of the stool sample to be examined as well as control specimens into the well of the microwell plate together with biotinylated anti-Entamoeba antibodies (Conjugate 1) for incubation at room temperature (20 - 25 °C). After a wash step, streptavidin poly-peroxidase conjugate (Conjugate 2) is added and it is incubated again at room temperature (20 - 25 °C). With the presence of Entamoeba antigens in a specimen, immobilized antibodies, the Entamoeba antigen, and the conjugated antibody form a sandwich complex. Another wash step removes the unattached streptavidin poly-peroxidase conjugate. After adding the substrate, the attached enzyme changes the colour of the previously colourless solution in the wells of the microwell plate to blue if the test is positive. Addition of a stop reagent changes the color from blue to yellow. The extinction is proportional to the concentration of Entamoeba antigens in the specimen.

4. Reagents provided

The reagents in the kit are sufficient for 96 determinations.

Plate	96	Microwell plate, 12 microwell strips (which can be divided) in strip holder, coated with specific monoclonal antibodies (mouse) to *Entamoeba histolytica* and *Entaboeba dispar*
Diluent | 1	100 ml	Sample dilution buffer, protein-buffered NaCl solution; ready for use; blue colored
Wash buffer	100 ml	Wash buffer, phosphate-buffered NaCl solution (concentrated 10-fold); contains 0.1 % thimerosal
Control | +	2 ml	Inactivated Entamoeba antigen; ready for use
Control | -	2 ml	Negative control (sample dilution buffer); ready for use
Conjugate | 1	13 ml	Biotin conjugated antibodies (mouse) to Entamoeba antigens in stabilized protein solution; ready for use, green colored
Conjugate | 2	13 ml	Streptavidin poly-peroxidase conjugate in stabilized protein solution; ready for use; orange colored
Substrate	13 ml	Hydrogen peroxide/TMB; ready for use
Stop	12 ml	Stop reagent; 1 N sulphuric acid; ready for use

Dangerous substances are indicated according to labelling obligations. For more details, refer to Safety Data Sheets (SDS) at www.r-biopharm.com.

5. Storage instructions

All reagents must be stored at 2 – 8 °C and can be used until the date printed on the label. Providing the diluted wash buffer is stored at 2 – 8 °C, it can be used for a maximum of 4 weeks. Microbial contamination must be prevented. After the expiry date, the quality guarantee is no longer valid.
The aluminum bag must be opened with scissors in such a way that the clip seal is not torn off. Any microwell strips which are not required must be returned to the aluminum bag and immediately stored at 2 – 8 °C.
The colorless substrate must also be protected from direct light to prevent it from decomposing or turning blue due to auto-oxidation. Once the substrate has turned blue, it must not be used.

6. Reagents required but not provided

6.1 Necessary reagents

The following reagents are required to perform the RIDASCREEN® Entamoeba test:

Reagents
Distilled or deionized water

6.2 Necessary laboratory equipment

The following equipment is required to perform the RIDASCREEN® Entamoeba test:

Equipment
Test tubes
Disposable pipettes (Article no.: Z0001)
Vortex mixer (optional, see 9.3.)
Micropipette for 50 - 100 µl and 1 ml volume
Measuring cylinder (1,000 ml)
Timer
Washing device for microtiter plates or multichannel pipette (300 µl).
Photometer for microtiter plates (450 nm, reference filter 620-650 nm)
Filter paper (laboratory towels)

7. Warnings and precautions for the users

For *in vitro* diagnostic use only.

This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories must be followed. Always adhere strictly to the user instructions for this test. Specimens or reagents must not be pipetted by mouth, and contact with injured skin or mucous membranes must be prevented. Wear personal safety gear (suitable gloves, laboratory coat, safety glasses) when handling the specimens, and wash hands after finishing the test. Do not smoke, eat, or drink in areas where samples are being processed.
For more details, refer to Safety Data Sheets (SDS) at www.r-biopharm.com.

The kit includes a positive control that contains the inactivated Entamoeba antigen. It must be treated as potentially infectious material and handled in accordance with the national safety regulations, just like the patient samples.

The wash buffer contains 0.1 % thimerosal as preservative. This substance must not be allowed to come into contact with skin or mucous membranes.

Ensure the proper and responsible disposal of all reagents and materials after their use. For disposal, please adhere to national regulations!

8. Collection and storage of specimens

Until it is used, store the test material at 2 - 8 °C. If the material cannot be used for a test within three days, we recommend storage at –20 °C or colder. Avoid freezing and thawing the specimen repeatedly. After diluting a stool sample in sample dilution buffer 1:11, it can be stored at 2 - 8 °C for use within seven days (Tab. 1).

Tab. 1: Specimen storage

Undiluted stool specimen		Diluted specimen
2 - 8 °C	≤ - 20 °C	2 - 8 °C
≤ 3 days	> 3 days	≤ 7 days

Stool samples and rectal smears should not be collected in transport containers which contain transport media with preservatives, animal sera, metal ions, oxidizing agents, or detergents since these may interfere with the RIDASCREEN® Entamoeba Test. If rectal smears are used, make sure that the volume of stool material is sufficient (approx. 100 mg) for the test.

Contact tracing should include stool samples taken from contact persons who do not exhibit clinical symptoms, in order to identify asymptomatic carriers.

9. Test procedure

9.1 General information

All reagents and the microwell Plate must be brought to room temperature (20–25 °C) before use. The microwell strips must not be removed from the aluminum bag until they have reached room temperature. The reagents must be thoroughly mixed immediately before use. After use, the microwell strips (placed in sealed bags) and the reagents must be stored at 2 - 8 °C. Once used, the microwell strips must not be used again. Reagents and microwell strips must not be used,if the packaging is damaged or the vials are leaking.
In order to prevent cross contamination, the samples must be prevented from coming into direct contact with the kit components.
The test must not be carried out in direct sunlight. We recommend covering the microwell plate or sealing with plastic wrap to prevent evaporation losses.

9.2 Preparing the washing buffer

Mix 1 part wash buffer concentrate Wash buffer with 9 parts distilled water. Any crystals present in the concentrate must be dissolved beforehand by warming in a water bath at 37 °C.

9.3 Preparing the specimens

Fill a labelled test tube with 1 ml RIDASCREEN® sample dilution buffer Diluent | 1.
Use a disposable pipette (article no. Z0001) to aspirate a sample of thin stool (approx. 100 µl) to just above the second bulge and add this to buffer in the test tube to make a suspension.
To suspend a solid stool sample, use an equivalent amount (approx. 50 - 100 mg) of the sample, handling it with a spatula or disposable inoculation loop.
Homogenize the stool suspension by aspiration into and ejection from a disposable pipette or, alternatively, blend in a Vortex mixer.

Let the suspension stand a short period of time for the coarse stool particles to settle; this clarified supernatant of the stool suspension can be used directly in the test. If the test procedure is carried out in an automated ELISA system, the supernatant must be particle-free. In this case, it is advisable to centrifuge the sample at 2,500 G for 5 minutes.

Note: Stool samples diluted in Diluent | 1 can be used in any other RIDASCREEN® ELISA, provided that it also uses Diluent | 1.

9.4 First incubation

After inserting a sufficient number of wells in the strip holder, add 100 µl of the positive control Control |+, the negative control Control |-, or the stool sample suspension to the wells. Subsequently add 100 µl of the biotin-conjugated antibody Conjugate | 1 and blend (by tapping lightly on the side of the plate); then incubate for 30 minutes at room temperature (20 - 25 °C).

9.5 Washing

Careful washing is important in order to achieve the correct results and should therefore proceed strictly according to the instructions. The incubated substance in the wells must be emptied into a waste container and discarded in accordance with local regulations. After this, knock out the plate onto absorbent paper in order to remove the residual moisture. Then wash the plate five times using 300 µl wash buffer Wash buffer each time. Make sure that the wells are emptied completely by knocking them out after each wash on a part of the absorbent paper which is still dry and unused.
If you use a microplate washer or fully automated ELISA, make sure that the machine is correctly adjusted; request settings from the manufacturer, if necessary. Appliances delivered by R-Biopharm are already programmed with validated settings and work protocols.
To avoid blocking the wash needles, only particle-free stool suspensions should be dispensed (see Item 9.3., Preparing the samples). Also make sure that all of the liquid is aspirated during each wash step.

9.6 Second incubation

Use a pipette to fill 100 µl streptavidin poly-peroxidase conjugate Conjugate | 2 into the wells, then incubate for 15 minutes at room temperature (20 – 25 °C).

9.7 Washing

Wash as described in Item 9.5.

9.8 Third incubation

Fill all wells with 100 µl substrate Substrate. Then incubate the plate for 15 minutes in darkness at room temperature (20 - 25 °C). Subsequently fill all wells with 50 µl stop reagent Stop in order
to stop the reaction. After blending cautiously by tapping lightly on the side of the plate, measure the extinction at 450 nm and at 620 nm reference wavelength.

Note: High-positive patient samples may cause black-colored precipitates of the substrate.

10. Quality control – indication of instability or detioration of reagents

For quality control purposes, positive and negative controls must be used each time the test is carried out, to ensure that the reagents are stable and that the test is conducted correctly. The test has been carried out correctly if the extinction rate (O.D.) for the negative control is less than 0.2 at 450 nm (less than 0.160 at 450/620 nm) and the measured value for the positive control is greater than 0.8 at 450 nm or at 450/620 nm. A value greater than 0.2 (0.160) for the negative control may indicate that washing was insufficient. Deviation from the required values, just like a turbid or blue coloring of the colorless substrate before it is filled into the wells, may indicate that the reagents have expired. If the stipulated values are not met, the following points must be checked before repeating the test:

- Expiry date of the reagents used
- Functionality of the equipment being used (e.g. calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks - a substrate solution which has turned blue must not be used.

If the conditions are still not fulfilled after repeating the test, please consult the manufacturer or your local R-Biopharm distributor.

11. Evaluation and interpretation

11.1. Calculating the cut-off

In order to establish the cut-off, 0.15 extinction units are added to the measured extinction for the negative control.

Cut-off = extinction for the negative control + 0.15

11.2. Test results

Assessment of the specimen is positive if the extinction rate is more than 10 % higher than the calculated cut-off value.

Assessment of the specimen is marginal if the extinction rate ranges from 10 % less to 10 % greater than the cut-off value. If the repeat examination with a fresh stool sample again falls within the gray zone, assessment of the sample is negative.

Samples with extinctions more than 10 % below the calculated cut-off must be considered negative.

12. Limitations of the method

The RIDASCREEN® Entamoeba Test identifies the antigens of *Entamoeba histolytica* and *Entamoeba dispar*. It is not possible to associate the determined level of extinction to the occurrence or severity of clinical symptoms. The results obtained must always be interpreted in combination with the clinical signs and symptoms.

A positive result does not rule out the presence of other infectious pathogens.

A negative result does not rule out the possibility of amoebiasis. Such a result may be due to intermittent excretion of the pathogen, or the amount of antigen in the sample may be too small. If the patient history supports a suspicion of *Entamoeba histolytica* infection, the examination should be repeated with another stool sample. If there is reason to suspect extraintestinal amoebiasis, the suspicion can be confirmed by the identification of specific antibodies to *Entamoeba histolytica* in serum (RIDASCREEN® E. histolytica IgG, Art. No.: K1721).

A marginal result may be due to non-homogeneous distribution of the antigens in the stool sample. In this case, examination should either be repeated with a second suspension from the same sample or another stool sample should be requested.

13. Performance characteristics

13.1 Analytical sensitivity

To determine the analytic sensitivity of the RIDASCREEN® Entamoeba ELISA, a linear dilution series from a sample with a known quantity of Entamoeba cysts was produced and then measured in triplicates. The limit of detection (LoD) is the last concentration to be evaluated as positive in all replicates. The results of that study are shown in Table 2.

Tab. 2: Analytical sensitivity results for *Entamoeba histolytica* in the RIDASCREEN® Entamoeba ELISA

	E. histolytica		***E. dispar***	
	MV [OD 450/620]	Cysts / Reaction	MV [OD 450/620]	Cysts / Reaction
LoD	0.173	17	0.200	595

13.2 Comparison with competitor product

Previously diagnosed stool samples (10 Entamoeba positive and 30 Entamoeba negative) were analyzed with the RIDASCREEN® Entamoeba Test and compared with the ELISA of a competitor. The results of that study are summarized in Table 3.

Tab. 3: Comparison of RIDASCREEN® Entamoeba ELISA and a competitor ELISA, using stool samples

		Competitor	
		positive	negative
RIDASCREEN® Entamoeba	positive	9	1
	negative	0	30

Positive agreement: 94.7 % negative agreement: 98.4 %

In addition, a serial dilution was produced from certified *E. histolytica* strains for a comparison study of RIDASCREEN® Entamoeba ELISA against two competitor products. The results are shown in Table 4.

Tab. 4: Comparison of RIDASCREEN® Entamoeba ELISA with two competitor ELISA, using a mixture of *Entamoeba histolytica* strains

E. histolytica	RIDASCREEN® Entamoeba MV [OD 450/620]	ELISA 1 MV [OD 450/620]	ELISA 2 MV [OD 450/620]
Strain HM1:IMSS			
1:10	2.428 **+++**	3.734 **+++**	2.533 **+++**
$1:10^2$	3.840 **+++**	3.566 **+++**	1.806 **+++**
$1:10^3$	3.885 **+++**	0.514 **-**	0.894 **++**
$1:10^4$	3.678 **+++**	0.015 **-**	0.313 **+**
$1:10^5$	2.528 **+++**	-0.003 **-**	0.208 **+**
$1:10^6$	0.890 **++**	-0.006 **-**	0.146 **+**
Strain HK9			
1:10	3.129 **+++**	3.493 **+++**	2.084 **+++**
$1:10^2$	3.594 **+++**	3.397 **+++**	1.498 **++**
$1:10^3$	3.683 **+++**	0.750 **++**	0.668 **++**
$1:10^4$	3.520 **+++**	0.050 **-**	0.251 **+**
$1:10^5$	1.695 **+++**	-0.004 **-**	0.152 **+**
$1:10^6$	0.696 **++**	-0.003 **-**	0.121 **-**
Strain 200:N1H			
1:10	2.269 **+++**	3.514 **+++**	2.654 **+++**
$1:10^2$	3.601 **+++**	3.464 **+++**	1.975 **+++**
$1:10^3$	3.638 **+++**	2.616 **+++**	1.245 **++**
$1:10^4$	3.672 **+++**	0.327 **+**	0.444 **+**
$1:10^5$	3.239 **+++**	0.055 **-**	0.236 **+**
$1:10^6$	0.996 **++**	0.003 **-**	0.138 **+**

OD450/620 ≥ 1.5: **+++** OD450/620 0.5 - 1.5: **++**

OD450/620 < 0.5: **+** OD450/620 < Cut-off: **-**

13.3 Cross-reactivity

A variety of pathogenic microorganisms from the intestinal tract were examined with the RIDASCREEN® Entamoeba Test and except for *Campylobacter coli*, they showed no cross reactivity. These studies were conducted with undiluted bacteria or virus suspensions shown to have concentrations of 10^6 to 10^9 organisms per ml. The results of that study are summarized in Table 5.

Tab. 5: Cross reactivity with pathogenic microorganisms

Organism	Origin	[OD 450/620] Mean value
Adenovirus	Cell culture supernatant	-0.002
Aeromonas hydrophila	Culture	-0.005
Arcobacter butzleri	Culture	0.008
Astrovirus	Cell culture supernatant	0.001
Bacillus cereus	Culture	0.003
Bacteroides fragilis	Culture	-0.005
Campylobacter coli	Culture	0.196
Campylobacter jejuni	Culture	0.017
Candida albicans	Culture	0.019
Citrobacter freundii	Culture	0.016
Clostridium difficile	Culture	0.042
Clostridium perfringens	Culture	0.006
Clostridium sordellii	Culture	0.007
Cryptosporidium muris	Culture	0.002
Cryptosporidium parvum	Culture	0.006
E. coli (O26:H-)	Culture	-0.002
E. coli (O6)	Culture	0.026
E. coli (O157:H7)	Culture	0.020
Enterobacter cloacae	Culture	0.002
Enterococcus faecalis	Culture	0.000
Giardia lamblia	Stool	0.004
Klebsiella oxytoca	Culture	0.032
Proteus vulgaris	Culture	0.003
Pseudomonas aeruginosa	Culture	0.002
Rotavirus	Cell culture supernatant	-0.003
Salmonella enteritidis	Culture	-0.004
Salmonella typhimurium	Culture	0.004
Serratia liquefaciens	Culture	-0.004
Shigella flexneri	Culture	0.018
Staphylococcus aureus	Culture	0.015
Staphylococcus epidermidis	Culture	-0.001
Vibrio parahaemolyticus	Culture	-0.002
Yersinia enterocolitica	Culture	0.001

13.4 Precision

To determine the intra-assay reproducibility, 40 replicates of these references were assayed, representing the complete measurement range from negative to high-positive. The mean values and the variation coefficients (VC) were determined for three lots of the kits. For the inter-assay reproducibility, references from ten different working days were assayed in duplicates, with two runs per day. The measurements were determined by three technicians for three lots of the kits. The inter-lot reproducibility was determined for all three lots of the kits. The results of that study are shown in Table 6.

Tab. 6: Reproducibility and precision of the RIDASCREEN® Entamoeba ELISA

Reference		Intra-assay			Inter-assay			Inter-lot
		Kit Lot 1	Kit Lot 2	Kit Lot 3	Kit Lot 1	Kit Lot 2	Kit Lot 3	Kit Lot 1-3
1	MV [OD 450/620]	1.901	1.402	1.465	1.581	1.707	1.507	1.598
	VC (%)	6.07 %	8.39 %	5.91 %	17.00 %	16.70 %	18.71 %	18.17 %
2	MV [OD 450/620]	1.375	1.095	1.209	1.155	1.253	1.124	1.177
	VC (%)	5.82 %	7.45 %	5.74 %	13.71 %	14.97 %	16.25 %	15.74 %
3	MV [OD 450/620]	1.091	0.976	0.810	0.933	1.010	0.869	0.937
	VC (%)	5.31 %	10.58 %	6.07 %	16.20 %	15.17 %	14.00 %	16.68 %
4	MV [OD 450/620]	0.606	0.532	0.512	0.507	0.556	0.461	0.508
	VC (%)	5.45 %	6.44 %	7.93 %	19.52 %	14.33 %	17.21 %	19.08 %
5	MV [OD 450/620]	0.350	0.325	0.204	0.306	0.330	0.276	0.304
	VC (%)	6.61 %	12.20 %	16.90 %	19.78 %	17.50 %	21.16 %	20.92 %
6	MV [OD 450/620]	-0.001	-0.002	0.000	0.012	0.001	0.005	0.006
	VC (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a

13.5 Interfering substances

The following list of substances showed no effects on the test results when they were blended into the supernatants of Entamoeba positive and Entamoeba negative stool samples in the described concentrations:

Mucin	5.0 % w/w	Diclofenac	0.1 % v/w
Human blood	5.0 % v/w	Cyclamate	1.3 % v/w
Pepto-Bismol	6.6 % v/w	Stearic acid / palmitinic acid	40 % w/w (1:1)
Loperamide	0.02 % w/w	Metronidazole	0.5 % solution

A possible dose effect relationship was investigated for barium sulfate (18.5 % w/w). This investigation of a serial dilution with barium sulfate, however, did not show a direct relationship between the concentration and the OD values. The only exception is the highest concentration that was tested, but it is even higher than the "worst case" concentration that was tested already in the first analysis (three times the daily dose). Interference due to barium sulfate can therefore be considered to be improbable.

14. Version history

Version number	Chapter and designation
2017-04-20	Previous version
2019-07-10	General revision 4. Reagents provided 8. Collection and storage of the specimens 9.2 Preparing the washing buffer 9.5 Washing 9.8 Third incubation 13.2 Comparison with competitor product

15. Explanation of symbols

General symbols

IVD	For in vitro diagnostic use
	Consult instructions for use
LOT	Lot number
	Expiry
	Store at
REF	Article number
	Number of tests
	Date of manufacture
	Manufacturer

Testspezifische Symbole

Plate	Microtiter plate
Diluent | 1	Sample dilution buffer
Wash buffer	Washing buffer
Control | +	Positive control
Control | -	Negative control
Conjugate | 1	Conjugate 1
Conjugate | 2	Conjugate 2
Substrate	Substrate
Stop	Stop reagent

16. References

1. Bracha, R. et al.: Differentiation of clinical isolates of Entamoeba histolytica by using spe-cific DNA probes. Clin. Microbiol. 28 (No. 4), 680 - 684 (1990).
2. Citronberg, R. J., Semel, J. D.: Severe vaginal infection with Entamoeba histolytica in a woman who recently returned from Mexico: Case report and review. Clin. Infect. Dis. 20, 700 - 702 (1995).
3. Espinosa-Cantellano, M., Martinez-Palomo, A.: Entamoeba histolytica: Mechanism of sur-face receptor capping. Exp. Parasitol. 79, 424 - 435 (1994).
4. Katzwinkel-Wladarsch, S. et al.: Direct amplification and differentiation of pathogenic and nonpathogenic Entamoeba histolytica DNA from stool specimen. Am. J. Trop. Med.-Hyg. 51 (1), 115 - 118 (1994).
5. Kean, B. H. et al.: Epidemic of amoebiasis and giardiasis in a biased population. Brit. J. Ven. Dis. 55, 375 - 378 (1979).
6. Mannweiler, E.: Immundiagnostik der Amöbiasis. Der Mikrobiologe 5. Jg., Heft 6, 194 - 200 (1995).
7. Ohnishi, K. et al.: Brain abscess due to infection with Entamoeba histolytica. Am. J. Trop. Med. Hyg. 51 (2), 180 - 182 (1994).
8. Petter, R. et al.: Characterization of two distinct gene transcripts for ribosomal protein L 21 from pathogenic and nonpathogenic strains of Entamoeba histolytica. Gene 150, 181 - 186 (1994).
9. Reed, Sh. L.: New Concepts regarding the pathogenesis of amebiasis. Clin. Infect. Dis. 21 (Suppl. 2), 182 - 185 (1995).
10. Strachan, W. D. et al.: Immunological differentiation of pathogenic and non-pathogenic isolates of Entamoeba histolytica. Lancet 12, 561 - 563 (1988).
11. van Lunzen, J., Tannich, E., Burchard, G.-D.: Amöbenruhr und Amöbenleberabszeß. Deutsches Ärzteblatt 93, Heft 2, 2659 - 2665 (1996).