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COMPARATIVE EVALUATION OF THE DIAGNOSTIC METHODS FOR DETECTION OF *GIARDIA INTESTINALIS* IN HUMAN FECAL SAMPLES

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ABSTRACT

AIM: To compared sensitivity and specificity of the light-microscopic morphodiagnostic technique, new commercial ELISA coproantigen test and immunochromatographic tests for the detection of *Giardia intestinalis* in human. METHODS: For *G. intestinalis* are tested 233 people with giardiasis and 30 individuals, negative for *G. intestinalis* - control group. Used are morphodiagnostic light microscopy of faeces (simple and concentrated native and colored Lugol`s iodine) in 233 and duodenal aspirate – in 32 people. In 178 individuals tested for *Giardia* coproantigen using RIDASCREEN® Giardia ELISA test; 60 samples using RIDA® Quick *Giardia* test; and 65 samples using RIDA® Quick *Cryptosporidium/Giardia* test. RESULTS: The sensitivity of the light-microscopic examination of smear of faecal samples, is 97,9 %; of duodenal aspirate – 86,5%; of smear concentrated faecal samples – 98,7%. Sensitivity of the ELISA test is 98,8%, of the immunochromatographic mono-*Giardia* test is 89,6% and immunochromatographic combi-*Cryptosporidium / Giardia* test is 92,9%, respectively. CONCLUSION: ELISA test for detection *Giardia* coproantigen is an alternative diagnostic means of morphodiagnostic light microscopy

Key words: *Giardia,* Giardiasis, light microscopy, coproantigen, ELISA, immunochromatographic tests.

INTRODUCTION

Giardia intestinalis is the most common protozoan enteric pathogen in humans, with an estimated global prevalence of 280 million cases per annum [1]. Giardiasis (ICD 10 - A.07.1) - infection with this parasite leads to malabsorption and diarrhoea in adults and children, but most often it occurs asymptomatic. Infections in children have been shown to have a negative impact on growth and development, and giardiasis has been recognized in 2004 as a 'neglected disease' by the World Health Organization [2]. After the events of September 11, 2001 *G. intestinalis* is included in the list of agents, Category B potential bioterrorism agents [3].

Giardia intestinalis is referred in developed countries as a re-emerging infectious agent [4].

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Epidemiological studies have shown that its prevalence varies between the population studied and geographically, from 2 to 5% in the industrialised world to 20 – 30% in the developing world and its transmission is enhanced in conditions where poor hygiene and sanitation. Even in some regions the spread reaches 50% and they talk about endemic giardiasis [5]

In Bulgaria, for the past 10 years prevalence of giardiasis average and less than 1%. In Stara Zagora region prevalence of giardiasis is below 1% [6, 7]. Detection of *Giardia intestinalis* is traditionally performed by microscopic examination of stool specimens. Repeating this examination once or twice on additional specimens improves the sensitivity of the test because of the intermittency of cyst excretion, time consuming, and requires high degree of client compliance [8, 9, 10, 11]. In addition, the required expertise is based on experience in examining *Giardia* cyst morphology by a light microscope and training in diagnostic microscopy requires extended periods of time. The sensitivity of morphodiagnostic technique

is approximately 46% on a single step due to the intermittent excretion of cysts over time, and at least three faecal samples have to be obtained over a 3-5 day period to achieve 94 % accuracy in positive *Giardia* diagnosis [11, 12, 13, 14].

Coproantigenic identification of *G. intestinalis* was introduced in the early 90's of last century. Today, new methods based on the detection of parasitic antigens offer valuable diagnostic alternatives for laboratories without personnel experienced with microscopy. Enzyme immunoassays (ELISA) is highly sensitive and specific for the diagnosis of giardiasis [15, 16, 17]. For these reasons, in the last years, different immunological methods have been developed as alternative methods for the diagnosis of giardiasis.

The aim of the present study we compared sensitivity and specificity of the light-microscopic morphodiagnostic technique, new commercial ELISA coproantigen test and immunochromatographic tests for the detection of *Giardia intestinalis* and/or *Cryptosporidium parvum* in human.

PACIENTS, MATERIALS AND METHODS

In the study we included 233 of the 483 patients positive for *G. intestinalis* and group of 30 persons negative for *G. intestinalis* – as control group. Positive faecal samples were obtained from patients with giardiasis. Male of the study was n=74 (31,7%), female was n=67 (28,8%); children (0-19) was n=141 (60,5%) and adults (20-78) was n=92 (39,5%). The symptoms of giardiasis was: diarrhea – in 134 (57.5%) patients, abdominal pain – in 120 (51.5%), vomiting – in 114 (48.9%), nausea – in 104 (44.6%), swelling and flatulence – in 101 (43.3%) anorexia - in 101 (43.3%), allergy - in 73 (31.3%).

We assume that the patient is actually positive if a triple-studies of various portions of faecal samples (or duodenal aspirate) are detected by light microscopy, cysts and/or trofozoits of *G. intestinalis*. If the result of the light microscopy, is negative, and coproantigen is identify by the other two methods, the patient undergoes a second series of tests to detect microscopic morphological forms of the *G. intestinalis.*

Sample collection

Fresh stool specimens (5-10 grams) were collected in clean plastic containers, and examined within 24 h from the disposal of faeces [18]. If specimens could not be tested within 24 h, they were stored in refrigerator at $+5^0$C, no later than 72 hours. The examination of duodenal aspirate was performed after fibrogastroduodenoscopy collected and incubated in glass tube at 37^0C up to 2 hours of its release.

The morphodiagnostic technique used in 233 patients with giardiasis, examined for the presence of *Giardia* infection using (1) light microscopy of faecal samples: 233 smears of faecal samples (native and colored with iodine); 32 native smears of the duodenal aspirate; light microscopy of concentrated faecal sample using formalin-ether sedimentation in 233 and zinc sulfate centrifugal flotation in 233 (2); 178 samples were examined for RIDASCREEN® Giardia ELISA coproantigen kit; (3) 60 samples using RIDA® Quick Giardia test; and 65 samples using RIDA® Quick Cryptosporidium/Giardia test.

Morphodiagnostic light microscopy technique

- *Light microscopy of faecal samples and duodenal aspirate*

For the native smear, a small sample of feces (or duodenal aspirate for throphozoites) was placed on a glass slide and mixed with a drop of 0,9% solutions of NaCl and the slide was covered with a glass coverslip and examined for the presence of parasites at 100× and 400× magnification. The same procedure mixed with a drop of of Lugol's iodine was added and examined for the presence of cysts of parasites at 100× and 400× magnification.

- *Formalin-ether sedimentation*

Approximately 5 g of faecal sample placed in a 15 ml glass, 10 ml of distilled water added and mixed with a glass rod with a vortex motion. Pipette 5 ml of the suspension placed in a centrifuge tube, added 6 ml 10% formalin in saline and 2 ml ether are added also. Centrifuged for three minutes 1500-2000 rpm. We made a smear of sediment on a slide, staining with Lugol 's solution and examined for the presence of parasites at 100× and 400× magnification.

- *Zinc sulphate (33% $ZnSO_4$) flotation*

Fresh faecal samples were involved mixing approximately 1 g of faeces with 9 ml of water in a 10 ml glass tube and homogenized with a glass rod with a vortex motion and the pellet resuspended in 9 ml of 33% $ZnSO_4$ and centrifuged at 2000 rpm for 3 min. A small

volume of faecal suspension was removed from the surface of the liquid using a wire loop and placed on a slide. The slide was examined for the presence of parasites at 100× and 400× magnification.
Giardia intestinalis cysts and throphozoits were identified by their typical size and morphology. In three patients were found vacuolar forms of *Blastocystis hominis*.

Immunodiagnostic technique
The test was performed according to manufacturer's instructions. Results were interpreted following the manufacturer's guidelines.

- *ELISA coproantigen test*

We tested for *Giardia* coproantigen using RIDASCREEN® *Giardia* ELISA test (r-Biopharm AG, Darmstadt, Germany). In the RIDASCREEN® *Gardia* test, a specific antibody is used in a sandwich-type method.

- *Immunochromatographic tests*
  - *RIDA®QUICK Giardia test*

The RIDA®QUICK *Giardia* test is for in vitro diagnostic use. The RIDA®Quick *Giardia* is a quick immunochromatographic test for the qualitative determination of *Giardia intestinalis* in stool samples.
The quick test is a single-step immunochromatographic lateral-flow test, where specific antibodies which are directed against *G. intestinalis* attach themselves to red latex particles. Other specific antibodies against the pathogen are firmly bound to the membrane. The stool sample is first suspended in the extraction buffer and then precipitated. An aliquot portion of the clear supernatant of the sample is placed on the test strip. The sample with the colored latex particles, to which antigen attach themselves if the test is positive, then pass through the membrane and is bonded to the specific catch band.

A maximum of two bands should appear, in the following order as seen from the sample-absorption site: one red test band and one blue control band. The following interpretations are possible: *Giardia* positive: the red and blue bands are visible; *Giardia* negative: only the blue band is visible; Not valid: no visible band or a combination other than the one described above or other changes in band color. Likewise, changes in band color which only appear after 10 minutes or later are also without any diagnostic value and must not be used for evaluation.
The results were interpreted in combination with the clinical picture. A positive result does not exclude the presence of another infectious pathogen. A negative result does not necessarily mean that there is no *Giardia intestinalis* infection. This is caused by intermittent excretion of the pathogen or by the quantity of antigens in the sample being too small.

-RIDA® Quick Cryptosporidium/Giardia Combi

The RIDA® Quick Cryptosporidium/Giardia Combi test is for in vitro diagnostic use. It is a quick immunochromatographic test for the qualitative determination of *Cryptosporidium parvum* and / or *Giardia intestinalis* in stool samples. The principle of this test is also based on the agglutination between *Cryptosporidium* and / or *Giardia* coproantigen and latex particles.

A maximum of three bands should appear in the following order, as seen from the sample-absorption site: One blue, one red and one green (control) band. If the green control band is missing, the test is invalid and cannot be evaluated!

The following interpretations are possible: *Cryptosporidia* positive: blue and green bands are visible; *Giardia* positive: red and green bands are visible; Cryptosporidium and *Giardia* positive: blue, red and green bands are visible; Negative: only the green band is visible; Not valid: no visible band or a combination other than the one described above or other changes in band color. Likewise, changes in band color which appear after 10 minutes or later are also without any diagnostic value and must not be used for evaluation.

Statistical evaluations were performed by the Student`s t-test (p-value>0,05). Proportions of positive samples and corresponding 95 % confidence intervals were calculated for each of the two tests.

RESULTS AND DISCUSSION
The sensitivity is the probability that the assay will be positive when the infection is present. The specificity is the probability that the assay will be negative when the infection is absent. The positive predictive value of a diagnostic test is the proportion of total positive test results that are truly positive. The negative predictive value of a diagnostic test is the proportion of total negative results that are

truly negative. They were calculated using the following formulas: sensitivity (%) = TP/(TP+FN) x 100 and specificity (%) = TN/(TN+FP) x 100 (TP : true positive, FN : false negative, TN : true negative and FP : false positive values). The positive predictive value of a diagnostic test is the proportion of total positive test results that are true positives. The negative predictive value of a diagnostic test is the proportion of total negative results that are true negatives. These were calculated using the following formulas : positive predictive value (%) = TP/(TP+FP) x 100 and negative predictive value (%) = TN/(TN+FN) x 100 (TP : true positive, FN : false negative, TN :true negative and FP : false positive values) [8].

The sensitivity of the light-microscopic examination of smear of faecal samples, is 97,9 %; of duodenal aspirate – 86,5%; of smear concentrated faecal samples – 98,7%. Sensitivity of the ELISA test is 98,8%, of the immunochromatographic mono-*Giardia* test is 89,6% and immunochromatographic combi-*Cryptosporidium / Giardia* test is 92,9%, respectively **(Table 1 and 2)**

Table 1. *Results of using diagnostic methods [in %]*

Diagnostic methods	**T P**	**Possitive for *G. intestinalis***	**T N**	**F P (n, %)**	**F N (n, %)**
Light microscopy of simple samples					
Smear of faecal samples	233	228	30	0	5
Smear of the duodenal aspirate	32	27	30	0	5
Light microscopy of concentrated faecal samples					
By formalin-ether sedimentation	233	230	30	0	3
By $ZnSO_4$ centrifugal flotation	233	230	30	0	3
***Giardia* coproantigenic identification**					
ELISA test	178	176	30	0	2
Immunochromatographic *Giardia* test	60	53	30	2	7
Immunochromatographic *Cryptosporidium / Giardia* test	65	60	30	1	5

*TP-true positive; TN-true negative; FP-false possitive; FN-false negative

Regnath T., et al., in Germany [19] and Tasic et al., in Serbia [20] conducted a similar study. Angelov I. et al., in Bulgaria compare efficacy of GSA 65 monoclonal antigen in stool specimens with efficacy of light morphodiagnostic microscopy (native

preparation colored with Lugol`s solution). They found that specificity of the ELISA test is 85,3% and sensitivity – 100% [21]. Papini et al., apply same rapid Cryptosporidium/Giardia immunochromatographic test for diagnosis of giardiasis in dogs and establish sensitivity of test 83% [8]. Conventional microscopy of three stool samples (with or without concentration techniques) is still being recommended as the reference standard ("golden standard") to diagnose infections caused by *G. intestinalis* [15]. Our study indicates that the test has a high sensitivity to ELISA (98,9%), and also that its specificity (100%) is high too. Sensitivity of Enzyme-linked immunosorbent assay was higher than that of the identification of *Giardia* immunoassay coproantigen ($p<0,05$). The sensitivity of the light-microscopic morfodiagnostic of a trofozoits of *G. intestinalis* in duodenal aspirate 86.5% is the lowest. Wolfe, 1992 also found that microscopic examination of duodenal aspirate is not highly sensitive method, but other authors as Konenkov, et al., 2006, Sergiev, et al., 2006, Agafonova et al., 2008, have the opposite opinion [11, 22, 23, 24]. Immunochromatographic tests have lower sensitivity and specificity and do not justify its high cost. The new RidaQuick assays are sensitive and specific for the detection of *G. intestinalis*. They are rapid to perform and do not require experienced staff or special technical equipment. Including the time for sample preparation, results are obtained within 10-15 min per test. The results of this study suggested that coprpantigenic diagnostic by

ELISA test is suitable for use in testing a larger number of samples, especially for screening persons in regions where *G. intestinalis* is a common wide pathogen. The RidaQuick assays are an alternative diagnostic means of screening stool samples, particularly for smaller and less well-equipped laboratories. Recent data on a similar assay with considerably less sensitivity than the assays tested by us indicate that these test systems need to be evaluated individually [25, 26].

Table 2. *The efficiency of using diagnostic tests [in %]*

Diagnostic tests	Sensitivity [%]	Specificity [%]	Positive predictive value [%]	Negative predictive value [%]
Smear of faecal samples	97,9	100	100	100
Smear of the duodenal aspirate	86,5	100	100	100
Concentration samples	98,7	100	100	100
ELISA test	98,9	100	100	100
Immunochromatographic Giardia test	89,6	93,8	96,8	93,8
Immunochromatographic Cryptosporidium / Giardia test	92,9	96,8	98,5	96,8

CONCLUSIONS

1. Enzyme-linked immunosorbent assay for coproantigenic detection of *G. intestinalis* has the highest sensitivity (98,9%), and 100%. It is quick and convenient method for screening tests.
2. Immunochromatographic tests have lower sensitivity and specificity. They are suitable for use in diarrhea and identification of *Cryptosporidium* coproantigen. In a positive test-band for *Cryptosporidium*, examination is necessary to continue with the light-microscopic examination.
3. Light microscopy of concentrated and colored Lugol`s solution preparation has so hight sensitivity (98,7%), and 100% sensibility. The positive sites of this examination are that besides morphological forms of *G. intestinalis* by light microscopy, a sample of faeces differentiates other intestinal parasites too. This leads to the detection of active owners of parasitism and there timely and proper conduct of etiological treatment.

To our knowledge, this is the first study in Bulgaria that uses the RIDASCREEN® *Giardia* ELISA coproantigenic test, RIDA®Quick *Giardia* test and RIDA® Quick *Cryptosporidium/Giardia*-Combi test to detect *Giardia* infection in humans and and compare their sensitivity and specificity.

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