

# LAMBLIA (GIARDIA) INTESTINALIS SPECIFIC GSA 65 ANTIGEN IN FECAL SAMPLES FROM CHILDREN WITH ASYMPTOMATIC LAMBLIOSIS AND ITS DIAGNOSTIC VALUE

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## Abstract

Stool specimens from 90 children were examined using two methods: with lugol's solution (for *Lamblia* [*Giardia*] intestinalis cysts) and ELISA - GSA 65 (for stool lamblia antigen). The specimens tested with lugol's solution were positive in 22 (24.4%) of the cases, and negative - in 68 (75.6%) cases. However, when the same samples were tested with GSA 65, it confirmed all positive results, and revealed other 10 positive cases in the group of 68 (14.7%) found as negative with the lugol's solution test. The results obtained illustrate a higher sensitivity and specificity of the ELISA test using monoclonal antibody to detect GSA 65.

**Key words:** *Lamblia* (*Giardia*) *intestinalis*, lugol's solution, lamblia antigen, GSA 65, immunodiagnosics

## INTRODUCTION

*Lamblia* (*Giardia*) [*L. (G.)*] *intestinalis* is still one of the most common intestinal protozoa found in children's contingents. Over the last five years, the average incidence of lambliosis in Bulgaria ranged from 1.03% to 1.34% (1, 2). The causing agent is isolated in two morphological forms: cystic form in asymptomatic carriers of the parasite, and vegetative form in the cases with clinical presentation and diarrhoeal symptoms.

In routine practice, the diagnosis is made by testing stool specimens with native preparation, stained with lugol's solution. The efficacy of the method is low: it depends on the way the specimens are collected and prepared for the investigation, and the number of times the specimens are tested. In addition, efficacy depends on the experience of the investigator. (3). The presence of *L. (G.)* *intestinalis* in the intestinal tract has oriented investigators to develop alternative methods to detect antigens of the parasite in bowel contents. Communications were published reporting the use of ELISA to prove the presence of lamblia antigen marked as GSA 65 (*Giardia* Specific Antigen) (4, 5, 6, 7). This makes it possible to diagnose carriers, irrespective of their clinical state, which is important in view of the fact that carriership without symptoms in lambliosis is common.

The aim of the study is to compare the efficacy of the method, proving the presence of GSA 65 antigen in stool specimens with the efficacy of a routine method of diagnosing lambliosis by using a native preparation stained with lugol's solution, detecting cyst of the parasite.

## ABBREVIATIONS USED IN THIS PAPER:

OD - Optical density GSA- *Giardia* Specific Antigen

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## MATERIALS AND METHODS

Stool specimens from 90 children aged 3 to 7 years were investigated. The specimens of 5-10 grams were placed in chemically clean containers. Each specimen was then concentrated by a formalin-ether method. Light microscopy was performed to detect intestinal protozoa by investigating of three native preparations prepared from the sediment and stained with lugol's solution. The result from the test was considered negative if no cysts of *L. (G.)* *intestinalis* were detected in a specimen tested three times.

The specimens were then divided into two groups, according to the results obtained from the test with lugol's solution, and were investigated for GSA 65. Group 1 included specimens positive for cysts of *L. (G.)* *intestinalis*, and group 2 - negative specimens.

The stool specimens of both groups were tested for fecal lamblia antigen using ELISA test "Giardia EZ Microplate Assay", according to instructions of the producer (REMEL Inc., USA). This test proves the presence of *L. (G.)* *intestinalis* GSA 65 specific antigen, and the result is measured by spectrophotometry or visually, by the intensity of coloration of the investigated and control specimens (5).

## Description of the method

The following steps were taken:

1. Stool specimens 200 µl, homogenized with a Specimen Dilution Buffer (buffered solution with rabbit serum and 0.02% trimezol) were pipetted into the wells, pretreated with anti-GSA 65 monoclonal antibodies. A positive control was added to one well and a negative - to the another. (Controls are provided with the test itself, and are prepared from human stool specimens, to serve as reagents and controls in the procedure.)
2. The specimens and controls were incubated at room temperature of 20°C for 60 min.
3. A threefold washing is performed with 10x concentrated buffered solution with 0.01% trimezol to remove the unbound material.
4. Enzyme conjugate (Peroxidase labeled monoclonal anti-GSA with 0.01% thimerosal) of 200 µl was added into each well, and the specimens were incubated at the same conditions for 30 min.
5. After a five-fold washing, 200 µl Color Substrate - TMB in buffer was added to each well.
6. The reaction was terminated after 10-minute incubation at 20°C, using 50 µl of a stop solution (1.0 N Sulfuric acid). Results were interpreted visually or spectrophotometrically at 450 nm wavelength. Visual interpretation was based on the intensity of yellow coloration of the wells examined, compared to the color table provided with the test. The result was considered positive for the presence of GSA 65 in the stool specimen if the yellow coloration intensity is at least 1+. A colorless reaction was considered negative, indicating the absence of GSA 65 in the stool specimen examined. Spectrophotometric interpretation was based on optical density (OD), measured in extinctions (E). If the difference between the values of OD for the specimens tested and the negative control was equal to greater than 0.05 E, it was assumed that specimens were positive for GSA 65. In case OD was less than 0.05 E, specimens were considered negative for GSA 65.

## RESULTS AND DISCUSSION

The study was carried on a group of 90 children, of which 36 girls (40%) and 54 boys (60%), aged 3-7 years. The results obtained are shown on Table 1.



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# **PROBLEMS**

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