A novel ELISA-based diagnostic test may replace the traditional microscopy in detection of *Blastocystis* spp. in human stool specimens

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Abstract

Objectives. *Blastocystis* is an enteric protozoan parasite highly prevalent in humans and animals. It is worldwide associated with non-specific symptoms, i.e. diarrhea, abdominal pain, anal itching, excess gas, and irritable bowel disease, and therefore under-diagnosed. Detection of *Blastocystis* is routinely performed by microscopy, culture, and formol-ethyl acetate concentration technique (FECT). Yet, these methods are laborious, require special skilled personnel, and time consuming. Since *Blastocystis* has several morphological forms (vacuolar, cyst, amoeboid, granular, multivacuolar, and avacuolar), microscopy is difficult. FECT destroys some of the forms during stool processing, therefore is unreliable. Culture requires 2–3 days for diagnosis and may allow preferential growth of specific strains while eliminating others. ELISA-based test for detection of *Blastocystis* antigens in fresh and preserved stool samples was recently launched and evaluated (CoproELISA® *Blastocystis,* Savyon, Israel). The aim of this work is to demonstrate the usefulness of the newly developed test, as a proper alternative to currently used methods, especially the microscopy.
Methods. A mixture of the most abundant human infecting strains was used to prepare polyclonal anti-*Blastocystis* antibodies, which compose the ELISA. A cohort of 179 fresh/frozen samples was tested by the newly developed ELISA, microscopy examination of Lugol's iodine staining, culture and staining with fluorescent (FITC) anti-*Blastocystis* antibodies (Antibodies Inc, USA). The culture and fluorescent antibodies results were considered as consensus for reference purposes.
Results. Considering the consensus results as reference, the ELISA performance demonstrates 82% sensitivity, 86% specificity, 84% accuracy, 82% PPV and 86% NPV. The sensitivity of Lugol staining microscopy was 18%. The ELISA detects the most prevalent subtypes in humans (1, 2, 3, and 5,), and all known morphological forms.
Conclusions. This work presents a unique ELISA test that provides superior performance compared to microscopy, the currently most widely used method. The ELISA enables high throughput screening, adaptation to automatic procedures and is overall cost-effective. In addition it is expeditious in providing reliable results and efficient requiring no special skilled personnel. Taken these considerations, the ELISA is expected to be the method of choice for diagnosis of *Blastocystis* in the common laboratory.

Introduction

Blastocystis is an enteric protozoan parasite highly prevalent in humans and animals. It is worldwide associated with non-specific symptoms, i.e. diarrhea, watery or loose stools, abdominal pain, weight loss, constipation, anal itching, excess gas, and irritable bowel disease. This wide array of non-specific symptoms has confounded the understanding of the potential pathogenicity of *Blastocystis* species. As a result, many of these infections are undiagnosed. Detection of *Blastocystis* is routinely performed by microscopy, culture, and formol-ethyl acetate concentration technique (FECT). Yet, these methods are laborious, require special skilled personnel, and are time consuming. Since *Blastocystis* has several morphological forms (vacuolar, cyst, amoeboid, granular, multivacuolar, and avacuolar), microscopy is difficult. FECT destroys some of the forms during stool processing, therefore is unreliable. Culture requires 2–3 days for diagnosis and may allow preferential growth of specific strains while eliminating others. Nevertheless, as of today, microscopy and culture are believed to be the “gold standard” methods for detection of *Blastocystis*. ELISA-based test for detection of *Blastocystis* antigens in fresh and preserved stool samples has recently been launched and evaluated (CoproELISA® *Blastocystis,* Savyon, Israel). This novel and unique ELISA is intended to be used for detection of *Blastocystis* antigens in human fecal specimens collected from patients with gastrointestinal symptoms. The test can be used for fecal specimens submitted for routine clinical testing from adults or children. In view of the apparent drawbacks of the currently used diagnostic methods for *Blastocystis* detection, the newly developed ELISA test demonstrates a proper alternative as a screening tool, and is expected to be the preferred method for the diagnosis of this pathogen in the clinical microbiology laboratory.

Methods

A mixture of the most abundant human infecting strains was used to prepare polyclonal anti-*Blastocystis* antibodies, which are used in the ELISA.
A cohort of 179 fresh/frozen and Formalin or SAF-fixed stool samples were collected at The University Hospital of Gazi, Turkey, and in Israel. The samples were tested by the newly developed ELISA, microscopy examination of Lugol's iodine staining, and culture. The samples collected in Turkey were also stained with fluorescent (FITC) anti-*Blastocystis* antibodies (Antibodies Inc, USA). Samples were characterized as positive or negative according to microscopy and/or culture. For samples characterized by fluorescent antibodies, the culture and fluorescent antibodies results were considered as consensus for reference purposes. ELISA results were measured for absorbance values at 450/620 and were defined according to calculated cut-off values as instructed in the kit insert.

Objective

The aim of this work is to demonstrate the usefulness of the newly developed ELISA test as a proper alternative to currently used methods in diagnosis of *Blastocystis*, especially the microscopic analysis

Results

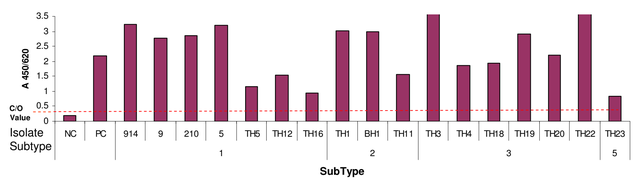
Table 1. Overall performance of the CoproELISA® *Blastocystis*

		Culture + FITC or Microscopy		
		POS	NEG	Total
CoproELISA® *Blastocystis* (Savyon)	POS	67	14	81
	NEG	6	92	98
	Total	73	106	179

Sensitivity: 83% **PPV: 92%**
Specificity: 94% **NPV:87%**
Accuracy: 89%

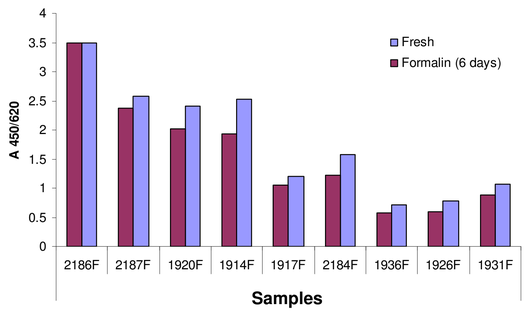
Objects: 179 (73 positives and 106 negatives) fresh/frozen and Formalin/SAF preserved samples collected in Turkey and Israel

Figure 1. Detection of most abundant *Blastocystis* subtypes in human by CoproELISA® *Blastocystis*



PC: positive control
NC: negative control

Figure 3. Comparison between detection of fresh/frozen Vs. Formalin/SAF preserved samples by CoproELISA® *Blastocystis*



Fresh/frozen and Formalin/SAF samples were compared after 6 days incubation in the respective conditions

Table 2. Comparison of CoproELISA® *Blastocystis* Vs. microscopy in consensus samples as defined by culture and fluorescent antibodies

		Culture + FITC		
		POS	NEG	Total
CoproELISA® *Blastocystis* (Savyon)	POS	32	7	39
	NEG	7	43	50
	Total	39	50	89

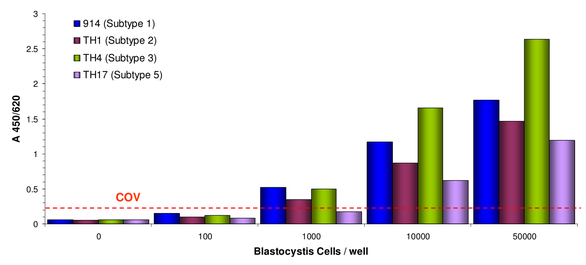
Sensitivity: 82% Specificity: 86% Accuracy: 84%
PPV: 82% NPV: 86%

		Culture + FITC		
		POS	NEG	Total
Microscopy	POS	7	0	7
	NEG	32	50	82
	Total	39	50	89

Sensitivity: 18% Specificity: 100% Accuracy: 64%
PPV: 100% NPV: 61%

Objects: 89 (39 positives and 50 negatives) frozen samples collected in Turkey

Figure 2. Analytical sensitivity of CoproELISA® *Blastocystis* in most abundant *Blastocystis* subtypes in human



Isolate	Subtype	LOD (cells/well)
914	1	230
TH1	2	380
TH4	3	250
TH17	5	620

Table 3. Cross reactivity with other commonly found gastroenteric pathogens in stool specimens

Organism	CoproELISA® *Blastocystis*
Blastocystis spp.	Positive
Endolimax nana	Negative
Entamoeba coli	Negative
Iodamoeba butschlii	Negative
Entamoeba histolytica/dispar	Negative
Dientamoeba fragilis	Negative
Entamoeba hartmanni	Negative
Cyclospora	Negative
Ascaris	Negative
Cryptosporidium spp.	Negative
Giardia lamblia	Negative
Hookworm	Negative
Balantidium Coli	Negative

Conclusions

- In view of the high prevalence of *Blastocystis* among a-symptomatic population, the performance of the ELISA provides the ground for using it as an efficient diagnostic tool
- The ELISA is proven to be much more efficient diagnostic tool than microscopy, which is currently the most common tool used for diagnosis of this pathogen in the clinical laboratory
- Taking the long time that culture demands and the special proficiency and tools that fluorescent test requires, the ELISA provides a cost-effective alternative to these methods as well
- The ELISA may be used for diagnosis in fresh/frozen as well as Formalin/SAF preserved samples in the same efficiency
- The ELISA detects the most prevalent subtypes in humans (1, 2, 3, 5), and most of the known morphological forms Cross reactivity study shows that The ELISA is highly specific and does not detect other pathogens that are common in gastroenteritis

Summary

- The newly developed ELISA is unique and provides superior performance compared to microscopy, the currently most widely used method
- The ELISA enables high throughput screening, adaptation to automatic procedures and is overall cost-effective
- The ELISA is expeditious and efficient in providing reliable results, requiring no special skilled personnel
- Taking overall considerations, the ELISA is expected to be the method of choice for diagnosis of *Blastocystis* in the common microbiology laboratory