

# ***Blastocystis hominis***

Enzyme-Linked Immunosorbent Assay  
(ELISA)

for the detection of *Blastocystis hominis* (*B. hominis*) cells  
in human feces

## **Instruction Manual**

Test kit for 96 determinations

Store at 2-8°C. **Do Not Freeze**

### **Savyon® Diagnostics Ltd.**

3 Habosem St. Ashdod 77610

ISRAEL

Tel. +972.8.8562920

Fax: +972.8.8523176

E-mail: support@savyondiagnosics.com

### **Intended Use**

The Savyon *B. hominis* test is an enzyme immunoassay for the rapid detection of the *B. hominis* cells in human fecal specimens. It is indicated for use with fecal specimens from patients with gastrointestinal symptoms to determine the presence of *B. hominis* infection. The test can be used for fecal specimens submitted for routine clinical testing from adults or children. Conventional microscopy is not a prerequisite for use of the test.

### **Introduction**

*Blastocystis hominis* (*B. hominis*) is an enteric protozoan parasite of humans and a variety of other animals (1,2). It has a worldwide distribution and is often the most commonly isolated organism in parasitological surveys (3). Early studies associated *B. hominis* infections with symptoms such as abdominal pain, diarrhea, constipation, fatigue, headaches, and depression (4). Subsequent reports added skin rash and joint pain to the list (5-7). The Center for Disease Control (CDC) states that the symptoms reported to be associated with blastocystosis infection are diarrhea, watery or loose stools, anal itching, abdominal pain, weight loss, and excess gas (CDC Fact Sheet (8)). 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 likely go undiagnosed. Detection of Blastocystis is routinely performed by methods such as microscopy, culture, and formyl acetate concentration technique (FECT). Yet, these methods all have flaws that make them

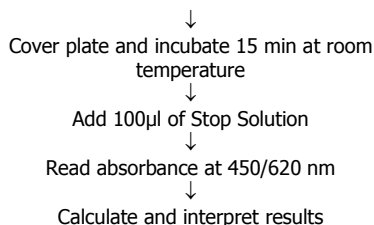
unreliable or time consuming. Since Blastocystis has several morphological forms (vacuolar, cyst, amoeboid, granular, multivacuolar, and avacuolar), microscopy is very difficult (1). In addition, FECT is unreliable because it destroys the multivacuolar, vacuolar, and granular forms of the parasite during stool processing (9). Culture requires 2-3 days to diagnosis and in some instances allows preferential growth of one subtype over another if more than one subtype is present in the stool (10). Nevertheless, microscopy and culture are believed to be the "gold standard" methods for detection of *B. hominis*.

### **Principle of the Test**

- Plates are coated with specific polyclonal antibodies directed against *B. hominis* antigens.
- Fecal sample to be tested is diluted in stool diluent and incubated with the pre-coated plate. In this step *B. hominis* antigens are bound to the immobilized antibodies.
- Non-specific Antigens are removed by washing.
- Anti-*B. hominis* conjugated to horseradish peroxidase (HRP) is added and incubated. In this step the HRP-conjugate is bound to the pre-bound antigen-antibody complex.
- Unbound conjugate is removed by washing.
- Upon the addition of TMB-substrate, the substrate is hydrolyzed by the peroxidase, yielding a blue solution of the reduced substrate.
- Upon the addition of the stop solution, the blue color turns yellow and should be read by an ELISA reader at a wavelength of 450/620 nm.
- The absorbance is proportional to the number of *B. hominis* cells present in the sample.

### **Summary of Procedure**

Wells of microtiter plate coated with anti-*B. hominis* antibodies  
↓  
Add 100 µl of Negative Control,  
100 µl of Positive Control, 100 µl of Cutoff Control  
(Cutoff: duplicate determination) and 100 µl of  
diluted specimens  
↓  
Cover plate and incubate 1h at 37°C at 100%  
humidity  
↓  
Wash 5 times with Wash Buffer (300 µl)  
↓  
Add 100 µl of HRP-Conjugate (Ready to Use)  
↓  
Cover plate and incubate 1 h at 37°C at 100%  
humidity  
↓  
Wash 5 times with Wash buffer (300 µl)  
↓  
Add 100µl of TMB-Substrate




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#### Kit contents:

##### Test Kit for 96 determinations:

1. **Microtiter plate coated with anti – *B. hominis* polyclonal antibodies:** 96 break-apart wells (8x12) coated with *anti – B. hominis* polyclonal antibodies, packed in an aluminum pouch containing a desiccant card. **1 plate**
2. **Concentrated Wash Buffer (20x):** A PBS -Tween buffer. **1 bottle, 100 ml**
3. **Stool Diluent (Blue):** A ready-to-use buffer solution. Contains less than 0.05% Proclin as preservative. **1 bottle, 70 ml**
4. **HRP-Conjugate (Green):** A ready-to-use solution containing Horseradish peroxidase (HRP) conjugated anti- *B. hominis* antibody. Contains less than 0.05% Proclin as preservative. **1 bottle, 15 ml**
5. **Positive Control:** A ready to use solution containing *B. hominis* antigen. Contains less than 0.05% Proclin as preservative. **1 vial, 1.5 ml**
6. **Cutoff Control:** A ready to use solution containing diluted *B. hominis* antigen. Contains less than 0.05% Proclin as preservative. **1 vial, 1.5 ml**
6. **TMB-Substrate:** A ready to use solution contains 3,3',5,5' tetramethylbenzidine as a chromogen and peroxide as a substrate. **1 bottle, 14 ml**
7. **Stop Solution:** A ready to use solution. Contains 1M H<sub>2</sub>SO<sub>4</sub>. **1 bottle, 15 ml**
8. **Plate cover:** **1 unit**
9. **Instruction Manual:** **1 unit**

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#### Materials Required But Not Supplied:

1. Clean test tubes for dilution of patients' stool.
  2. Adjustable micropipettes, or multichannel pipettes (5-50, 50-200 and 200-1000µl ranges) and disposable tips.
  3. Disposable plastic/wooden collectors or teaspoons.
  4. One-liter volumetric flask.
  5. One 50 ml volumetric cylinder.
  6. Wash bottle.
  7. Absorbent paper.
  8. Vortex mixer.
  9. A 37°C water bath with a lid, or a moisture chamber placed in a 37°C incubator.
  10. ELISA-reader equipped with 450/620 nm filters.
  11. Distilled or double de-ionized water.
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#### Warnings and Precautions

1. Reagents should be brought to room temperature before use.
2. When handling assay wells, avoid scratching the bottom of the wells because this may result in elevated absorbance readings.
3. Stool samples, microassay wells, micropipette tips and disposable stool collectors and tubes should be handled and disposed of as potential biohazards after use. Wear gloves when doing the test.
4. **Unused microassay wells must be replaced in the resealable pouch with the desiccant to protect them from moisture.**
5. TMB-Substrate solution is an irritant material to skin and mucous membranes. Avoid direct contact.
6. Diluted sulfuric acid (1M H<sub>2</sub>SO<sub>4</sub>) is an irritant agent for the eyes and skin. In case of contact with eyes, immediately flush area with water and consult a physician).

#### Storage and Shelf-Life of Reagents

1. The expiration date of the kit is given on the label. Expiration dates for each component are listed on individual labels. The kit should be stored between 2° and 8°C and should be returned to the refrigerator as soon as possible after use. Exposure of originally stoppered or sealed components to ambient temperature for a few hours will not cause damage to the reagents. **DO NOT FREEZE!**
2. Unused strips must be resealed in the aluminum pouch with the desiccant card, by rolling the open end and sealing tightly with tape over the entire length of the opening.

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#### Stool Collection

Standard collection and handling procedures used in-house for fecal specimens for culture are appropriate. The test is compatible with specimens that were fixed in 10% formalin or in Sodium Acetate Formalin (SAF) but not in polyvinyl alcohol (PVA) fixative. Specimens should be transported as soon as possible and stored between 2° and 8°C. If possible, fresh stool samples should be tested within 48 hours after collection. Store specimens at -20°C, or lower, if the test cannot be performed within 48 hours. Freezing and thawing of the specimen, especially multiple times, may result in loss of activity due to degradation or proteolysis of the antigens.

#### Procedure

1. Set up one dilution tube for each specimen to be tested. 1.5 mL Eppendorf tubes are

recommended for this purpose. Add 400  $\mu$ L *Stool Diluent* to each tube. *Label the tube.*

**2. Thoroughly mix (vortex) the fecal specimen to ensure adequate sampling.**

3. For **formed samples**, use a wooden collector or a disposable teaspoon to transfer the fecal specimen to the tube. Coat the collector completely before transferring the specimen. Mix the collector in the *Stool Diluent* to remove as much sample as possible and squeeze the collector against the side of the tube to express any residual liquid. This procedure results in the transfer of approximately 0.15 to 0.20 g of specimen. If a disposable teaspoon is used for formed specimens, transfer approximately 0.15 to 0.20 g of specimen (about the size of a small pea) to the *stool Diluent*. For **liquid samples**, transfer 400  $\mu$ L specimen to tube. Make sure the liquid specimens are evenly suspended (vortexed).

4. Let the tube stand for at least 10 minutes but not more than 30 minutes until large particulate matter is precipitated (decantation). Use upper liquid phase for testing. **DO NOT USE CENTRIFUGE FOR THIS PURPOSE**

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## Test Procedure

### A. Preparation of Reagents

1. Bring all components and clinical specimens to be tested to room temperature. Determine the total number of specimens to be tested. In addition to the specimens, the following must be included in each test: one well of Negative Control (*Use Stool Diluent for this purpose*), one well of Positive Control and one well of Cutoff Control.
2. Withdraw the microtiter plate from its aluminum pouch by cutting one end near the seal. Leave the required number of strips (according to the number of specimens to be tested) in the 96 well frame.
3. Dilute the Concentrated Wash Buffer 1/20 with double-deionized or distilled water. For example, in order to prepare one liter of Wash Buffer, add 50ml of the Concentrated Wash Buffer to 950ml of double-deionized or distilled water.

### B. Incubation of stool samples and controls

4. Pipette or dispense 100 $\mu$ L of Positive control (~2 drops), Negative Control (*Stool Diluent*), Cut-off control (Cutoff control: duplicate determination) and pre-diluted stool into separate wells of the test strip.
5. Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.
6. Discard the liquid content of the wells.

7. **Washing step:** Fill each well with Wash Buffer up to the end of the well and discard the liquid, repeat this step **FIVE** times. If using an automatic washing machine, it is advised that the first two washes will be performed manually to avoid clogging the washing machine with stool particulate matter.

8. Dry the strips and frame by gently tapping them over clean absorbent paper.

### C. Incubation with Conjugate

11. Pipette or dispense 100 $\mu$ L of ready-to-use conjugate into each well (~2 drops).
12. Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.
13. Discard the liquid content and wash **FIVE** times as described in steps 8-9.

### D. Incubation with TMB Substrate

15. Pipette or dispense 100 $\mu$ L (~2 drops) of TMB-Substrate into each well, cover the strips with a plate cover and incubate at room temperature for **15 minutes**.
16. Stop the reaction by adding 100 $\mu$ L (~2 drops) of Stop Solution (1M H<sub>2</sub>SO<sub>4</sub>) to each well.

### E. Determination of Results

17. Determine the absorbance at 450/620 nm and record the results. Determination should not exceed 30 minutes following stopping of chromogenic reaction.

**Note:** Any air bubbles should be removed before reading. The bottom of the ELISA plate should be carefully wiped

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### Test Validation

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

1. **Positive Control:** The absorbance value should be  $\geq 1.0$  at 450/620 nm.
2. **Cut-off Control:** The average absorbance value of the cut-off Control performed in duplicate determination should be  $0.3 \leq \text{cut-off} \leq 0.6$  at 450/620 nm.
3. **Positive control / Cut-off Control Ratio** should be  $\geq 2$
4. **Negative Control:** The absorbance value should be  $\leq 0.3$  at 450/620 nm.

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### Determination of Cut-Off Value and Interpretation of Results

1. The cut-off value (COV) is determined by the average absorbance at 450/620 nm of the Cutoff Control run in duplicate.
2. Samples that present absorbance values lower than the COV should be considered

negative and samples that exhibit absorbance values higher than the COV should be considered positive.

### Test Limitations

Positive result does not exclude the presence of other etiologies. It is therefore advised to take into account all clinical and laboratory data before making final diagnosis and decide upon appropriate patient management.

### Performance Characteristics of the Test

In one study performed in-house at Savyon diagnostics, a total of 34 stool specimens were tested for presence of *B. hominis* by culture and further evaluated by coproELISA *B. hominis* test. The results of this evaluation are shown in Table 1:

Table 1.

		CoproELISA <i>B. hominis</i>	
		Positive	Negative
Culture	Positive	18	1
	Negative	0	15

Sensitivity: 94.7%  
 Specificity: 100%  
 PPV: 100%  
 NPV: 93.8%

In a second study performed at a reference laboratory in the US, a total of 127 formalin- or SAF-fixed stool samples were tested by coproELISA *B. hominis* test. The presence of gastrointestinal parasites in these specimens was pre-determined by microscopic examination. The results of this evaluation are shown in Table 2:

Table 2.

		CoproELISA <i>B. hominis</i>	
		Positive	Negative
Microscopy	Positive	49	2
	Negative	3	73

Sensitivity: 96.1%  
 Specificity: 96.1%  
 PPV: 94.2%  
 NPV: 97.3%

In this study, stool samples harboring other parasitic organisms (as validated by microscopic examination) were tested by coproELISA *B. hominis*. No cross reactivity was observed with the following enteric parasites: *G. lamblia*, *Cryptosporidium spp.*, *I. Butschlij*, *E. vermicularis*, *C. cayetanensis*, *D. fragilis*, *E. histolytica/dispar*, *E. nana*, *E. hartmanni*, *T. trichiura*, *E. coli* & *B. coli*

### Precision

Intra-assay (within-run) precision of the test is shown in Table 3 below:

Table 3.

Sample	No. of Replicates	Mean Value	CV%
Positive	8	1.255	2.75
Negative	8	0.066	6.25

Inter-assay (between-run) precision of the test is shown in Table 4 below:

Table 4.

Sample	No. of Replicates	Mean Value	CV%
Positive	8	1.305	4.15
Negative	8	0.087	8.25

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