Crypto / Giardia Ag Combo (Fecal) ELISA
Cat # 8310-3

<table>
<thead>
<tr>
<th>Test</th>
<th>Crypto/ Giardia Combo ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>ELISA: Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>Principle</td>
<td>Sandwich ELISA: Antibody Coated Plate</td>
</tr>
<tr>
<td>Detection Range</td>
<td>Qualitative - Positive, Negative and Cut-off</td>
</tr>
<tr>
<td>Sample</td>
<td>Stool sample</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>99%</td>
</tr>
<tr>
<td>Total Time</td>
<td>~100 min</td>
</tr>
<tr>
<td>Shelf Life</td>
<td>12 months</td>
</tr>
</tbody>
</table>

*Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*
INTENDED USE
This ELISA is an in vitro immunoassay for the qualitative determination of Giardia and Cryptosporidium antigen in feces.

SUMMARY AND EXPLANATION
Giardia lamblia is the protozoan parasite responsible for the disease giardiasis. Symptoms of acute giardiasis include diarrhea, nausea, weight loss, malabsorption, abdominal cramps, flatulence and anemia. The disease may manifest itself as an acute, chronic or as an asymptomatic infection. Giardiasis is the most prevalent parasitic disease in the United States and is responsible for an estimated 100 million mild infections and 1 million severe infections each year.

The mode of transmission of Giardia is through fecal-oral ingestion of cysts. Epidemics of giardiasis have been documented in day care centers and by drinking contaminated water. Day care centers may be directly or indirectly responsible for 45% of diagnosed Giardia infections in the United States. One study found 54% of the children at a day care center were infected.

Another important source of Giardia infection is among homosexual men. Prevalence rates of 5 to 19% for this population have been reported.

Cryptosporidium is a coccidian parasite that is recognized as an important enteric pathogen. The organism causes an acute, though self-limiting infection in immunocompetent individuals. Incubation periods of 1 to 12 days have been reported with most oocyst shedding ending by day 21. Symptoms range from mild to severe diarrhea with a variety of complications.

The infection in immunocompromised patients is much more severe and may often be life threatening. Passage of fluid, up to 12 liters per day, has been reported.

Multiple pathways of Cryptosporidium transmission have been implicated. These include animal to human, water contamination and person-to-person. The latter may include contact between members of the same household, day care centers, and homosexual men.

Diagnosis of Giardia and Cryptosporidium infections has been done through a number of invasive and non-invasive techniques. Of the non-invasive techniques, microscopic examination of stools has been the most common. However, this method relies on an experienced technician and subsequent observation of intact organisms. Because of the historically low proficiency of correct microscopic examinations and intermittent excretion of organisms, alternative diagnostic methods have been investigated.

One important alternative has been the development of an antigen capture enzyme linked immunosorbent assay (ELISA) for use with stools. These tests have shown comparable sensitivity to experienced microscopic examinations, are fairly simple to perform and do not require the observation of intact organisms.

PRINCIPLE OF PROCEDURE
During the first incubation, Giardia and/or Cryptosporidium antigens present in stool specimens are captured by antibodies attached to the microwells. The wells are incubated and washed before anti-Giardia and anti-Cryptosporidium antibodies conjugated to peroxidase are added. The enzyme conjugate will “sandwich” any antigen bound to the wells. After washings to remove any unbound enzymes, a Chromogen is added, which develops a blue color in the presence of the enzyme complex. The Stop Solution ends the reaction and turns the blue color to yellow. If no antigen is captured, or if there is an insufficient level of antigen, no colored reaction will take place.
REAGENTS

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Strips</td>
<td>Microwells containing anti-\textit{Giardia} and anti-\textit{Cryptosporidium} antibodies - 96 test wells in a strip holder.</td>
<td>MT PLATE</td>
</tr>
<tr>
<td>Enzyme Conjugate</td>
<td>One (1) bottle containing 11 ml of peroxidase labeled anti-\textit{Giardia} and anti-\textit{Cryptosporidium} antibodies with Thimerosal.</td>
<td>CONJ</td>
</tr>
<tr>
<td>\textit{Giardia} Positive Control</td>
<td>One (1) vial containing 2 ml of a diluted \textit{Giardia} positive formalinized stool supernatant.</td>
<td>CONTROL +</td>
</tr>
<tr>
<td>\textit{Cryptosporidium} Positive Control</td>
<td>One (1) vial containing 2 ml of a diluted \textit{Cryptosporidium} positive formalinized stool supernatant</td>
<td>CONTROL +</td>
</tr>
<tr>
<td>Negative Control</td>
<td>One (1) vial containing 2 ml of a G/C negative formalinized stool supernatant.</td>
<td>CONTROL −</td>
</tr>
<tr>
<td>Chromogen</td>
<td>One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB) and peroxide.</td>
<td>SUBS TMB</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>Four (4) bottles containing 30 ml of a buffered protein solution with Thimerosal.</td>
<td>SPECM DIL</td>
</tr>
<tr>
<td>Wash Concentrate (20X)</td>
<td>Two (2) bottles containing 25 ml of concentrated buffer with surfactant and Thimerosal.</td>
<td>WASH BUF</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>One (1) bottle containing 11 ml of 1 M phosphoric acid.</td>
<td>SOLN</td>
</tr>
</tbody>
</table>

WARNINGS/PRECAUTIONS

For In Vitro Diagnostic Use

Do not use solutions if they precipitate or become cloudy.

Exception: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.

Do not add azides to the samples or any of the reagents.

Controls and some reagents contain Thimerosal as a preservative.

Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.

Stop solution is a 5% solution of phosphoric acid in water. If spilled on the skin, wash with copious amounts of water. If acid gets into the eyes, wash with copious amounts of water and seek medical attention.

Persons who are color blind or visually impaired may not be able to read the test visually and should use spectrophotometer readings to interpret results.

STORAGE CONDITIONS

Reagents, strips and bottled components:

Store between 2-8 °C.

Squeeze bottle containing diluted wash buffer may be stored at room temperature.

PREPARATIONS

Wash Buffer – Remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.
COLLECTION OF STOOL (FECES)

No modification of collection techniques used for standard microscopic O&P examinations is needed. Stool samples may be used as unpreserved or frozen, or in preservation media of 10% formalin, SAF or MF.

Unpreserved samples should be kept at 2-8 ºC and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20 ºC or lower until used. Freezing does not adversely affect the test.

Formalized, SAF and MF preserved samples may be kept at room temperature (15-25 ºC) and tested within 18 months of collection. DO NOT freeze preserved samples.

All dilutions of unpreserved stools must be made with the Dilution Buffer provided.

PREPARATION OF SAMPLE

Fresh/Frozen Stools

Thaw sample if needed. Prepare a 1:4 dilution in tubes using 0.3 ml of Dilution Buffer and one swab of fecal specimen (approximately 0.1 g). Coat swab with specimen and transfer into the Dilution Buffer, expressing as much liquid as possible and mix well. For watery specimens, add 0.1 ml of sample to 0.3 ml Dilution Buffer in tubes.

Preserved Stools (Formalin, SAF, and MF)

Mix contents thoroughly inside collection container. No further processing is required. If needed, prepared samples can be centrifuged at 2000-3000 g for 5-10 minutes. Ensure supernatant is clear before use.

PROCEDURE

Materials Provided

*Giardia/Cryptosporidium* Stool Antigen Microwell ELISA Kit

Materials Required But Not Provided

Transfer Pipettes

Squeeze bottle for washing strips (narrow tip is recommended)

Graduated Cylinder

Reagent grade (DI) water

Micropipette

Suggested Equipment

ELISA plate reader with 450 and 620-650 nm filters

Proper Temperature

All incubations are at room temperature (15 to 25 ºC)

TEST PROCEDURE

1. Break off the required number of wells needed (number of samples plus 3 for controls) and place in holder.
2. Using a micropipette, add 100 µl of negative control to well # 1, 100 µl of *Giardia* positive control to well # 2 and 100 µl of *Cryptosporidium* positive control to well # 3.*
3. Using a micropipette, add 50 µl of Dilution Buffer to each sample well. DO NOT add Dilution Buffer to control wells.

DAI Code # 3
4. Add 50 µl of sample to each well with Dilution Buffer.
5. Incubate for 60 minutes at room temperature (15-25 ºC), then wash.** After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
6. Add 2 drops of Enzyme Conjugate to each well.
7. Incubate for 30 minutes at room temperature (15-25 ºC), then wash.** After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
8. Add 2 drops of Chromogen to each well.
9. Incubate for 10 minutes at room temperature (15-25 ºC).
10. Add 2 drops of Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
11. Read results visually or at 450/620-650 nm. Zero reader on air.

* Controls must be included each time the kit is run.
** Washings consist of vigorously filling each well to overflowing and decanting contents five (5) separate times.

RESULTS
Interpretation of Results - Visual
Reactive: Any sample well that is obviously more yellow than the negative control well.
Non-reactive: Any sample well that is not obviously more yellow than the negative control well.

NOTE: The negative control, as well as some samples, may show some slight color. A sample well must be obviously darker than the negative control well to be called a positive result. Please refer to the enclosed visual read card for color comparisons.

Interpretation of Results - ELISA Reader
Zero reader on air. Read all wells at 450/620-650 nm.
Reactive: Absorbance reading of 0.08 OD units and above indicates the sample contains Giardia or Cryptosporidium antigen.
Non-reactive: Absorbance reading less than 0.08 OD units indicates the sample does not contain detectable levels of Giardia or Cryptosporidium antigen.

LIMITATION OF PROCEDURE
Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.
DO NOT concentrate stool samples. Assay will not give accurate results on a concentrated sample. A negative result can occur from an antigen level lower than the detection limits of this assay. Multiple samples over time may be indicated for those patients that are suspected of being positive for Giardia or Cryptosporidium.

EXPECTED VALUES
Normal healthy individuals should be free of Giardia and Cryptosporidium and should test negative. A positive reaction indicates that the patient is shedding detectable amounts of either antigen.
SPECIFIC PERFORMANCE CHARACTERISTICS

Study #1

<table>
<thead>
<tr>
<th>Reference Method*</th>
<th>DAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>35</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

Positive Agreement: 100% (35/35)
Negative Agreement: 100% (95/95)

<table>
<thead>
<tr>
<th>Reference Method*</th>
<th>DAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>52</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

Positive Agreement: 100% (52/52)
Negative Agreement: 99% (95/96)

<table>
<thead>
<tr>
<th>Reference Method*</th>
<th>DAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>11</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

Positive Agreement: 100% (11/11)
Negative Agreement: 99% (95/96)

*Reference Method refers to a commercially available ELISA.

QUALITY CONTROL
The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must have an absorbance of at least 0.5 OD units and the negative control must be less than 0.08 OD units. Should the value fall below this limit, the kit should not be used.

TROUBLESHOOTING
Problem: Negative control has substantial color development.
Correction: Washings were insufficient. Repeat test with more vigorous washings

CROSS REACTIVITY
No cross-reactions were seen with the following organisms:
Entamoeba hartmanni, Endolimax nana, Entamoeba histolytica/dispar, Entamoeba coli, Blastocystis hominis, Dientamoeba fragilis, Chilomastix mesnili, Strongyloides stercoralis, Ascaris lumbricoides, Enterobius vermicularis, Diphyllobothrium species, Hymenolepis nana, Clonorchis sinensis, Enteromonas hominis, Trichuris trichiura, Iodamoeba buetschlii, Hookworm, Schistosoma mansoni, rotavirus, Taenia
eggs, *Fasciola* eggs, *Isospora belli*, *Entamoeba polecki*, adenovirus, & 33 bacterial species (list available on request).

**REFERENCES**