DRG® Cryptosporidium Ag (stool) (EIA-3467)

Revised 23 Sept. 2010 rm (Vers. 4.0) **Not for sale in the USA**

Please use only the valid version of the package insert provided with the kit.

Intended Use

This ELISA is an *in vitro* immunoassay for the qualitative determination of *Cryptosporidium* antigen in fecal specimens.

Summary and Explanation

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. [1,8,9,10,11,13]

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. [1,2,3,12,14,16]

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. [1,2,12,14,16]

Diagnosis of *Cryptosporidium* infections was done originally by direct detection techniques. Of these, microscopic examination of stools using stains or fluorescence labeled antibodies 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, alternative diagnostic methods have been investigated. [4,5,16,17]

One important alternative has been the development of an antigen capture enzyme linked immunosorbent assay (ELISA) for use with stools. These tests, which have shown comparable sensitivity to experienced microscopic examinations, are fairly simple to perform and do not require the observation of intact organisms.[6,7]

Principle of Procedure

During the first incubation, *Cryptosporidium* specific antigen present in the stool specimens are captured by antibodies attached to the microwells. The wells are incubated and washed before anti-*Cryptosporidium* antibodies conjugated to peroxidase are added. The enzyme conjugate will "sandwich" any antigen bound to the wells. After washings to remove unbound enzyme, 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

Item	Description	Symbol
Test Strips	Microwells containing anti-*Cryptosporidium* antibodies - 96 test wells in a test strip holder.	MT PLATE
Enzyme Conjugate	One (1) bottle containing 11 ml of perozidase labeled anti-*Cryptosporidium* antibodies with thimerosal.	CONJ
Positive Control	One (1) vial containing 2 ml of a diluted *Cryptosporidium* positive formalinized stool supernatant.	CONTROL +
Negative Control	One (1) vial containing 2 ml of a *Cryptosporidium* negative formalinized stool supernatant.	CONTROL −
Chromogen	One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB) and peroxide.	SUBS TMB
Wash Concentrate (20X)	Two (2) bottles containing 25 ml of concentrated buffer with detergent and thimerosal.	WASH BUF
Dilution Buffer	Four (4) bottles containing 30 ml of a buffered protein solution with thimerosal.	SPECM DIL
Stop Solution	One (1) bottle containing 11 ml of 1 M phosphoric acid.	SOLN

Warnings/Precautions

- **Do not deviate from the specified procedures when performing this assay**. All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
- For In Vitro Diagnostic Use Only.
- Do not interchange reagents between kits with different lot numbers.
- Do not use reagents that are beyond their expiration dates. Expiration dates are on each reagent label. Use of reagents beyond their expiration dates may affect results.
- Unused microwells should be stored in the desiccated pouch to protect them from moisture.
- 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, which may be irritating to skin, eyes and mucous membranes. In case of contact, flush eyes or rinse skin with copious amounts of water.
- 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 spectrophotometric readings to interpret results.

Storage Conditions

Reagents, strips and bottled components should be stored at 2-8°C.
Squeeze bottle containing diluted wash buffer may be stored at room temperature.

Preparation

Before use, bring all reagents and samples to room temperature (15-25°C) and mix.
(20X) Wash Concentrate may precipitate during refrigerated storage, but will go back into solution when brought to room temperature (15-25°C) and mixed. **Ensure that (20X) wash concentrate is completely in solution before diluting to working concentration**. To dilute (20X) wash concentrate to working dilution, 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, in Cary-Blair Transport Medium or in preservation media of 10% formalin or SAF.
Unpreserved samples should be kept at 2-8°C and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20°C or lower until used. Avoid multiple freeze/thaw cycles.
Formalized and SAF preserved samples may be kept at room temperature (15-25°C) or at 2-8°C and tested within 18 months of collection. DO NOT freeze preserved samples.
Samples in Cary-Blair should be kept at 2-8°C or -20°C and tested within 1 week of collection. Avoid multiple freeze/thaw cycles.

All dilutions of stools must be made with the Dilution Buffer provided. Do not use dilution buffer from a kit with a different lot number.

Procedure

Materials Provided

- Cryptosporidium Antigen Stool ELISA Kit
- Microplate strip holder

Materials Required But Not Provided

- Transfer Pipettes
- Squeeze bottle for washing strips (narrow tip is recommended)
- Graduated Cylinder
- Reagent grade (DI) water
- Micropipette
- Applicator sticks (recommended) or swabs for sample preparation
- Sample dilution tubes

Suggested Equipment

ELISA plate reader with 450 and 620-650 nm filters

Proper Temperature

All incubations are at room temperature (15 to 25°C)

Test Procedure

Notes*:*

- Ensure all samples and reagents are at room temperature (15-25°C) before use. Frozen samples must be thawed completely before use.
- If needed, prepared samples can be centrifuged at 2000-3000 g for 5-10 minutes. Ensure supernatant is clear before use.
- When running the assay, try to avoid the formation of bubbles in the wells. Bubbles may affect overall performance and reading of end results. Slapping the wells out on a clean absorbent towel after each wash step should help to minimize bubbles in the wells.
- **Specimens can be run following the *In Well Dilution Procedure* or the *In Tube Dilution Procedure*. See below for specific instructions on how to run the assay using each procedure.**

In Well Dilution Procedure:

1. Break off the required number of wells needed (number of samples plus 2 for controls) and place in holder.
2. Prepare sample dilutions in tubes using **0.3 ml** of Dilution Buffer and **0.1 g**, about the size of a small pea, of fecal sample using an applicator stick. Mix thoroughly before using.
   - IF USING SWABS, add **0.6 ml** of dilution buffer to dilution tube. Coat the swab with a thin layer of specimen and mix into dilution buffer, expressing as much fluid as possible. Mix thoroughly before using.
3. **For watery unpreserved specimens**, mix contents then add **0.1 ml** of sample to **0.3 ml** Dilution Buffer in dilution tubes. Mix thoroughly before using.
4. **For samples in SAF, 10% Formalin or Cary-Blair,** mix contents thoroughly inside container. No further processing is required.
5. Using a micropipette, add **100 µl** of negative control to well # 1 and **100 µl** of positive control to well # 2.
6. Using a micropipette, add **50 µl** of Dilution Buffer to each sample well. **DO NOT add Dilution Buffer to control wells.**
7. Add **50 µl** of sample to each sample well with Dilution Buffer.
8. Incubate for **60 minutes** at room temperature (15-25°C), then wash.***** After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer. **Proceed to step 9.**

In Tube Dilution Procedure:

1. Break off the required number of wells needed (number of samples plus 2 for controls) and place in holder.

2. Prepare sample dilutions in tubes using **0.7 ml** of Dilution Buffer and **0.1 g**, about the size of a small pea, of fecal sample using an applicator stick. Mix thoroughly before using.
- IF USING SWABS, add **1ml** of dilution buffer to dilution tube. Coat the swab with a thin layer of specimen and mix into dilution buffer, expressing as much fluid as possible. Mix thoroughly before using.

3. **For watery unpreserved specimens**, mix contents then add **0.1 ml** of sample to **0.7 ml** of Dilution Buffer in dilution tubes. Mix thoroughly before using.
4. **For samples in SAF, 10% Formalin or Cary-Blair**, mix contents then add **0.2 ml** of sample to **0.3 ml** of Dilution Buffer in dilution tubes. Mix thoroughly before using.
5. Using a micropipette, add **100 µl** of negative control to well # 1.
6. Using a micropipette, add **100 µl** of positive control to well # 2.
7. Add **100 µl** of sample to each well.
8. Incubate for **60 minutes** at room temperature (15-25°C), then wash.***** After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer. **Proceed to step 9.**
9. Add **2 drops** of Enzyme Conjugate to each well.
10. Incubate for **30 minutes** at room temperature (15-25°C), then wash.***** After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
11. Add **2 drops** of Chromogen to each well.

12. Incubate for **10 minutes** at room temperature (15-25°C).
13. Add **2 drops** of Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger for approximately **15 seconds**. Read reaction within **5 minutes** after adding stop solution.
14. Read results visually or a dual wavelength of 450/620-650 nm. Zero reader on air.

**** Washings consist of vigorously filling each well to overflowing and decanting contents five (5) separate times. When possible, avoid formation of bubbles in the wells as this may affect the end results.***

Quality Control

The positive and negative control must be included each time the assay is run. The use of a positive and negative control allows easy validation of kit stability.
Negative control should appear colorless when read visually and should read less than 0.08 O.D. when read at a dual wavelength of 450/620-650 nm.
Positive control should be a clearly visible yellow color and read at greater than 0.5 O.D. when read at a dual wavelength of 450/620-650 nm.

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 O.D. units and above indicates the sample contains *Cryptosporidium* antigen.
Non-reactive: Absorbance reading less than 0.08 O.D. units indicates the sample does not contain detectable levels of *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 Cryptosporidium.

Expected Values

Normal healthy individuals should be free of *Cryptosporidium* and should test negative.
A positive reaction indicates that the patient is shedding detectable amounts of *Cryptosporidium* antigen. Certain populations, such as homosexual men and children in day care settings, have shown higher rates of infection with *Cryptosporidium* than the normal population.
Please refer to the Summary section for references.

Performance Characteristics

Study 1:

A study was performed with the IVD Research, Inc. *Cryptosporidium* assay using fresh/frozen specimens, specimens preserved in 10% Formalin and SAF and specimens in Cary-Blair Transport media. There were a total of 94 specimens used in the study that were confirmed positive or negative for *Cryptosporidium* by microscopy. Of the 94 specimens there were 16 that were positive for *Cryptosporidium*, and 78 that were negative for *Cryptosporidium.* The results from the study are shown in the following table.

		Microscopy	
		+	-
Cryptosporidium EIA-3467	+	16	0
	-	0	78

Sensitivity: 100% (16/16)
Specificity: 100% (78/78)

Study 2:

Another study was performed comparing the IVD Research, Inc. *Cryptosporidium* assay with another commercially available ELISA. The study was performed using fresh/frozen specimens and specimens preserved in 10% Formalin and SAF. There were a total of 94 specimens used in the study that were confirmed positive or negative for *Cryptosporidium*.

Of the 94 specimens there were 16 that were positive for *Cryptosporidium,* and 78 that were negative for *Cryptosporidium.* The results from the study are shown in the following table.

		EIA-3467.	
		+	-
Other Commercial ELISA	+	16	0
	-	0	78

Positive Agreement: 100% (16/16)
Negative Agreement: 100% (78/78)

Reproducibility
The intra-assay (well to well) CV was calculated using 4 positive and 4 negative samples assayed 24 times in a single run. The mean CV was 5.96% with the highest being 9.83%.

The inter-assay (run to run) CV was calculated using 4 positive and 4 negative samples assayed on three separate days. The mean CV was 4.48% with the highest being 7.3%.

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, Giardia lamblia, Rotavirus, Taenia eggs, Fasciola eggs, Isospora belli, Entamoeba polecki, Adenovirus, & 33 bacterial species (list available on request).

Troubleshooting

Problem: Negative control has excessive color after development.
Reason: Inadequate washings
Correction: Wash more vigorously. Remove excessive liquid from the wells by tapping against an Absorbent towel. Do not allow test wells to dry out

Bibliography

1. Chapman, P.A. "Cryptosporidiosis: Recent Trends in Epidemiology, Diagnosis, and Treatment." Serodiag & Immunother Infect Dis #2, 1988, pp. 311-317.
2. Meyer, E.A. "Waterborne *Giardia* and *Cryptosporidium*." Parasit Today. Vol. 4, #7, 1988, pp. 200-201.
3. Garcia, L., Bruckner, D., Brewer, T., "Cryptosporidiosis in Patients with AIDS." ACPR, May 1988, pp. 38-41.
4. Stibbs, H., Ongerth, J. "Immunofluorescence Detection of *Cryptosporidium* Oocysts in Fecal Smears." J Clin Micro, Vol 24 #4, Oct. 1986, pp.517-521.
5. McLaughlin, J. et al. "Indentification of *Cryptosporidium* Oocysts by Monoclonal Antibody." Lancet, January 3, 1987, pp.51.
6. Ungar, B. "Enzyme-Linked Immunoassay for Detection of *Cryptosporidium* Antigens in Fecal Specimens." J Clin Micro, Vol. 28 #11, Nov 1990, pp. 2491-2495.
7. Anusz, K., et al. "Detection of *Cryptosporidium parvum* Oocysts in Bovine Feces by Monoclonal Antibody Capture Enzyme-Linked Immunosorbent Assay." J. Clin Micro, Vol. 28 #12, dec. 1990, pp. 2770-2774.
8. Jokipii, L., et al. "*Cryptosporidium*: A Frequent Finding In Patients With Gastrointestinal Symptoms." Lancet, August 13, 1983, pp. 358-360.
9. Shephard, R., et al. "Shedding of Oocysts of *Cryptosporidium* in Immunocompetent Patients." J Clin Pathol, Vol. 41, 1988, pp. 1104-1106.
10. Holten-Anderson, W., et al. "Prevelence of *Cryptosporidium* Among Patients with Acute Enteric Infection." J. Infect, Vol. 9, 1984, pp. 277-282.
11. Jokipii, L. and Jokipii, M. "Timing of Symptoms and Oocyst Excretion in Human Cryptosporidiosis." N Engl J Med, Vol. 315 #26, 1986, pp.1643-1647.
12. Egger, M., et al. "Symptoms and Transmission of Intestinal Cryptosporidiosis." Arch Dis Child, Vol 65, pp 445-447.
13. Hart, M., et al. "Acute Self-Limited Colitis Associated with *Cryptosporidium* in an Immunocompetent Patient." J Ped Gastro Nutr, Vol. 8, 1989, pp. 401-403.
14. Nwanyanwu, O., et al. "Cryptosporidiosis in a Day-Care Center." Texas Med, Vol. 85, June 1989, pp. 40-43.
15. Sloan, L.M., and Rosenblatt, J.E. "Evaluation of Enzyme-Linked Immunosorbent Assay for Detection of *Cryptosporodium* spp. in Stool Specimens." J Clin Micro, Vol. 31 #6, June 1993, pp. 1468-1471.
16. Current, W. and Garica, L. "Cryptosporidiosis." Clin Micro Rev, Vol. 4 #3, July 1991, pp. 325-358.
17. Weber, R. et al. "Threshold of Detection of *Cryptosporidium* Oocysts in Human Stool Specimens; Evidence for Low Sensitivity of Current Diagnostic Methods." J Clin Micro, Vol. 29 #7, July 1991, pp. 1323-1327.

Revised 23 Sept. 2010 rm (Vers. 4.0) **Not for sale in the USA**

Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità