



**PARA-TECT™**  
**Cryptosporidium Antigen Detection**  
**Microwell ELISA Directions For Use**  
**For In Vitro Diagnostic Use**

Catalog # MCC-CP-96, 96 Test

### Intended Use

This microwell enzyme-linked immunoabsorbant assay (ELISA) detection kit (*Cryptosporidium* ELISA Kit) is an in vitro diagnostic (IVD) immunoassay for the detection of *Cryptosporidium* species antigen in human feces using peroxidase as the indicator enzyme. The assay may be read visually or with an ELISA reader. Concentrated fecal samples cannot be used with this immunoassay. Rather, this IVD *Cryptosporidium* ELISA Kit is intended to be used with stools that are fresh, frozen or preserved in 10% formalin, SAF or Medical Chemical Corporation's (MCC) Universal fixative in a clinical laboratory use setting.

### 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.<sup>1,8,9,10,11,13</sup>

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.<sup>1,2,3,12,14,16</sup>

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.<sup>1,2,12,14,16</sup>

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.<sup>4,5,16,17</sup>

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.<sup>6,7</sup>

### Principle of Procedure

This ELISA is an in vitro immunoassay for the qualitative determination of *Cryptosporidium* species antigen in human feces. The assay uses rabbit anti-*Cryptosporidium* polyclonal antibodies to capture the species antigen from the stool supernatant. A second set of goat anti-*Cryptosporidium* polyclonal antibodies are then added which sandwiches the captured species antigen. This reaction is visualized by the addition of anti-goat polyclonal antibodies conjugated to peroxidase and the chromogen tetramethylbenzidine (TMB). The resulting blue color development is converted to an easily read yellow color by addition of an acidic "stop solution" to end the reaction. The presence of yellow color above 0.15 OD absorbance indicates presence of *Cryptosporidium* oocysts or species antigens.

### Reagents

Test strips: microwells containing anti-*Cryptosporidium* polyclonal antibodies - 96 test wells.

Test strip holder: One (1).

Reagent 1: One (1) bottle containing 11 mL of goat anti-*Cryptosporidium* antibodies with blue dye and Thimerosal.

Reagent 2: One (1) bottle containing 11 mL of anti-goat-peroxidase with red dye and Thimerosal.

Positive control: One (1) vial containing 2 mL of a diluted *Cryptosporidium* positive formalinized stool supernatant.

Negative control: One (1) vial containing 2 mL of a *Cryptosporidium* negative formalinized stool supernatant.

Chromogen: One (1) bottle containing 11 mL of the chromogen tetramethylbenzidine (TMB) and peroxide.

Wash Concentrate 20X: Two (2) bottles containing 25 mL of concentrated buffer and surfactant with Thimerosal.

Stop solution: One (1) bottle containing 11 mL of 1 M phosphoric acid

## 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 test visually and should use spectrophotometric readings to interpret results.

Do not use kit past the expiration date.

## Storage Conditions

Reagents, strips and bottled components: store between 2 - 8° C.

Squeeze bottle containing diluted wash buffer may be stored at room temperature for up to one year.

Wash Buffer - Remove cap and add contents of one bottle of Wash Concentrate (20X) to a squeeze bottle containing 475 mL of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings. Working wash buffer may be stored at room temperature (15-25° C) for up to one (1) year.

## Specimen Collection and Preparation

### 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 MCC's Universal Fixative.

Fresh 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 -15° to -25° C or lower until used. Freezing does not adversely affect the test.

Preserved samples (formalin, SAF and MCC's Universal Fixative) may be kept at room temperature (15-25° C) and tested within 6 months of collection. DO NOT freeze preserved samples.

All dilutions of unpreserved stools must be made with the diluted wash buffer.

### Preparation of Fresh/Frozen Stools

Thaw sample if needed. Add sufficient diluted wash buffer to make approximately a 1:4 dilution (1 gram or a pea size of fecal sample to 3 mL of diluted wash buffer) and mix well.

### Preserved of Preserved Stools (Formalin, SAF and Universal Fixative)

Mix contents thoroughly inside collection container. No further processing is required.

## Materials For Procedure

### Materials Provided

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

### Suggested Equipment

ELISA plate reader with 450 and 620-650 nm filters

## Test Procedure

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

1. Break off the required number of wells needed (number of samples plus 2 for controls) and place in holder.
2. Add 2 drops (approximately 100 µL) of negative control to well # 1 and 2 drops of positive control to well # 2.
3. Add 2 drops of the stool supernatant to each test well. Mix wells by tapping plate gently for 30 seconds.
4. Incubate for 30 minutes at room temperature (15-25° C). Shake out contents of wells into discard container, then wash.\*
5. Add 2 drops of Reagent 1 (blue solution) to each well.
6. Incubate for 5 minutes at room temperature (15-25° C). Shake out contents of wells into discard container, then wash.\*
7. Add 2 drops of Reagent 2 (red solution) to each well.
8. Incubate for 5 minutes at room temperature (15-25° C). Shake out contents of wells into discard container, then wash.\*
9. Add 2 drops of Chromogen to each well.
10. Incubate 5 minutes at room temperature (15-25° C).
11. Add 2 drops of Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
12. Read results visually or at 450/620-650 nm within 60 minutes after adding Stop Solution. Zero reader on air.

\* Washings consist of vigorously filling each well to overflowing and decanting contents three separate times. After the third fill, decant the contents into a designated discard container, turn the plate upside down and slap dry against paper towel covered solid surface. Controls must be included each time the kit is run.

## Interpretation of Results

### Interpretation of Results - Visual (Manual)

**Reactive:** Any sample well that is obviously more yellow than the negative control well.

**Non-reactive:** Any sample well that does not have obvious and significant yellow color.

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 0.15 OD units and above indicates the sample contains *Cryptosporidium* species antigen.

**Non-reactive:** Absorbance reading less than 0.15 OD units indicates the sample does not contain detectable levels of *Cryptosporidium* species antigen.

## Reporting of Results

The majority of *Cryptosporidium* species infections are believed to be from *C. parvum*, although it has recently been reported that non-*parvum* species have, in fact, been found in human stools.<sup>1,2,12,14,16,18,19</sup> In addition, it is not known how many of the tested samples used to validate this test might have been non-*parvum* species, because it is not known whether the reagents of this *Cryptosporidium* ELISA immunoassay will react with any non-*parvum* species. However, it is known that this *Cryptosporidium* ELISA immunoassay is quite specific to the Genus: *Cryptosporidium*. Thus, we recommend that positive results not be reported as “*Cryptosporidium parvum* antigen positive”, nor as “*C. parvum* antigen positive”, since only the Genus and not the species has been accurately established. Instead, we recommend that all positive results be reported as “***Cryptosporidium* species antigen positive**” or as “***Crypto. species antigen positive***”.

## Expected Values & Analytical Sensitivity

This assay has an analytical sensitivity [i.e., detection limit] of approximately 50 nanograms of soluble protein antigen per mL of *Cryptosporidium* species antigen or 10 oocysts per well. Below these concentrations, the optical absorbance falls below 0.15 OD units when read bichromatically at 450 nm and 620-650 nm, and a negative result should be reported.

Normal healthy individuals should be free of *Cryptosporidium* and should test negative, that is, an OD value of less than 0.15 absorbance reading. A positive reaction, at 0.15 OD units and above, indicates that the patient is shedding detectable amounts of *Cryptosporidium* species 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.

## Expected Specificity

No cross-reactions were seen with the following organisms:

*Entamoeba hartmanni*, *Endolimax nana*, *Entamoeba histolytica/dispar*, *Entamoeba coli*, *Blastocystis hominis*, *Dientamoeba fragilis*, *Chilomastix mesnili*, *Giardia lamblia*, *Cyclospora cayetanensis* oocysts, *Strongyloides stercoralis*, *Ascaris lumbricoides*, *Enterobius vermicularis* (pinworm), *Diphyllobothrium* species, *Hymenolepis nana*, *Clonorchis sinensis*, *Enteromonas hominis*, *Trichuris trichiura*, *Iodamoeba buetschlii*, Hookworm (*Necator americanus*), *Schistosoma mansoni*, *Taenia* eggs, *Fasciola* eggs, *Isospora belli*, *Entamoeba polecki*, adenovirus, rotavirus, *E. coli*, *Campylobacter*, *Salmonella*, *Shigella*, *Yersinia* and 29 other bacterial species (list available on request).

## Specific Performance Characteristics

### Study #1 (Outside Lab)

A total of 174 formalin, SAF, fresh/frozen and Universal Fixative preserved stools were tested against the ELISA test. The results were interpreted visually. The following results were obtained.

	Micro + (O&P)	Micro – (O&P)
IVD ELISA +	24	4
IVD ELISA -	0	146
Sensitivity: 100% (24/24)		95% CI = 86% to 100%
Specificity: 97% (146/150)		95% CI = 93% to 99%

### Study #2 (Manufacturer Performed)

A total of 44 fresh or fresh/frozen stools examined by O&P microscopy and/or another commercial ELISA were tested against this IVD ELISA. The following results were obtained.

	ELISA	ELISA
IVD ELISA +	14	0
IVD ELISA -	0	30
Positive Agreement = 100% (14/14)		
Negative Agreement = 100% (30/30)		

The breakdown for the samples in the above studies is as follows:

Media	<u><i>Cryptosporidium</i> +</u>	<u>Other Parasites</u>
Fresh/Frozen	14	0
10% Formalin	24	25
SAF	1	83
MCC	2*	31

\*These two samples were seeded samples.

## Precision

Two sites performed the assay using the same lot of kits and the same samples three times over a three-day period. The following results were obtained:

Well	Lab #1			Lab #2		
	Mean	Std Dev	%CV	Mean	Std Dev	%CV
1 – Neg Cntrl	.02	.03	146	.01	.00	62
2 – Pos Cntrl	1.41	.09	7	1.34	.06	4
3 – Pos	.80	.10	13	.72	.08	12
4 – Pos	1.15	.13	11	1.13	.10	9
5 – Pos	.96	.10	11	1.08	.10	9
6 – Pos	.31	.13	40	.35	.06	17
7 – Pos	1.27	.16	12	1.30	.11	9
8 – Pos	.39	.04	11	.39	.05	14
9 – Pos	.24	.03	12	.25	.04	16
10 – Neg	.01	.00	45	.01	.00	30
11 – Neg	.01	.01	185	.01	.01	183
12 – Neg	.00	.00	20	.03	.01	47
13 – Neg	.01	.00	20	.01	.00	10
14 – Neg	.01	.00	42	.01	.01	98
15 – Neg	.01	.01	95	.02	.00	22
16 – Neg	.01	.00	35	.01	.00	18

## Limitation of Procedure

Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.

Assay is intended for use with human stools only. *It should not be used for non-human applications.*

**DO NOT concentrate stool samples.** Assay will not give accurate results on a concentrated sample. Samples with fecal fat have not been tested in this assay.

A negative result can occur from a species 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*.

## 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 0.15 OD units. Should the value fall below this limit, the kit should not be used. Call technical support for further instructions.

## Troubleshooting

**Problem:** Negative control has substantial color development.

**Correction:** Washings were insufficient. Repeat test with more vigorous washings

## References

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**Revised September 2007**

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