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# **ProSpecT™ Giardia EZ Microplate Assay**

**REF** R2458596 ......

EN

### **INTENDED USE**

ProSpecT Giardia EZ Microplate Assay uses a monoclonal antibody for the qualitative detection of Giardia Specific Antigen (GSA 65) in aqueous extracts of faecal specimens.

# SUMMARY

Giardiasis is now recognized as an important human intestinal disease in most areas of the world. The causative organism, Giardia lamblia, is the most frequently identified protozoan parasite in stool specimens submitted to U.S. public health laboratories<sup>3</sup>. This parasite has been implicated in a number of epidemics<sup>4,5</sup> and the endemicity in the U.S. is well recognized.

Prevalence in adults is estimated at 4-7%8. Higher prevalence rates have been reported in children<sup>1,15</sup> and in homosexual males<sup>6,7</sup>. Acute symptoms of giardiasis may include diarrhoea, malabsorption, abdominal cramps, anorexia, nausea, weight loss, flatulence, anaemia, and general weakness lasting from several weeks to several months<sup>16</sup>. Chronic infections can also occur with or without an acute phase, are often associated with treatment failure, and may result in recurrent symptoms. Infection with Giardia may also be asymptomatic<sup>2</sup>.

Giardia Specific Antigen (GSA 65) is a macromolecule that has been found in association with Giardia infections and has been used as the basis of immunoassays<sup>9,10,11,12</sup>. GSA 65 is a 65,000 molecular weight glycoprotein that is produced in abundant quantities by the Giardia lamblia protozoa as they multiply within the host intestinal tract. The antigen is present only when Giardia infection is present and it is possible to find GSA 65 in stool specimens without visible signs of cvsts or trophozoites<sup>9,10,11</sup>. GSA 65 is a Giardia Specific Antigen and anti-GSA 65 antibodies have not been found to cross react with other enteric parasites 1,10 GSA 65 is stable to transport through the host intestinal tract as well as to most routine procedures used to collect and transport stool specimens for ova and parasite (O&P) microscopic examination<sup>1,11,12,1</sup>

### PRINCIPLE OF THE TEST

ProSpecT Giardia EZ Microplate Assay is a solid phase immunoassay for the detection of GSA 6514. The break-away microplate wells are coated with anti-GSA 65 antibody. The enzyme conjugate (monoclonal anti-GSA antibody labelled with horseradish peroxidase enzyme) and diluted stool specimens are simultaneously incubated in the well. If GSA 65 is present, it is 'captured' between the anti-GSA 65 antibody on the wells and

the enzyme conjugate. After incubation, the wells are washed to 6.5. remove excess specimen and any unbound enzyme conjugate. The substrate for the enzyme, 3,3',5,5'-tetramethylbenzidine (TMB), is added. In a positive reaction, the enzyme bound to the well by GSA 65 converts the substrate to a coloured reaction product. Colour development can be detected visually or spectrophotometrically. In a negative reaction, there is no GSA 65 or an insufficient level of GSA 65 present to bind the enzyme conjugate and no coloured reaction product develops.

# SYMBOL DEFINITIONS

REF IVD Σ

In Vitro Diagnostic Medical Device Contains sufficient for <n> tests

Catalogue Number



Consult Instructions for Use (IFU)



Temperature Limitation (Storage Temp.)



Batch Code (Lot Number) Use By (Expiration Date)



Manufacturer

DILUTED SAMPLE Diluted Sample

# KIT CONTENTS, PREPARATION FOR USE AND $_{6.13}$ .

The ProSpecT Giardia EZ Microplate Assay includes sufficient reagents to perform  $\checkmark$  96 tests.

# See also Precautions, section 6.

The expiration date of each kit is stated on the package label. Store all components at 2 to 8°C.

Before use, bring all reagents to room temperature (20 - 25°C)

and mix gently. Return the unused reagents to the refrigerator

All reagents, except the Wash Buffer, are supplied at working strength. Reagents can be dispensed directly from the dropper bottles or poured out for use with multichannel pipettes. If excess reagent has been poured, the excess should be discarded. Do not pour excess reagent back into the bottle.



Instructions for Use **Transfer pipettes** 

Microplate Strip Holder and Cover **Procedure Card** 

# MICROTITRATION PLATE Microplate\* (8 wells / strip)

12 strips coated with rabbit anti-GSA 65

antibody. Unused microplate strips should be stored in the foil pouch containing desiccant to exclude moisture.

# CONJUGATE

# **Enzyme Conjugate\***

One dropper bottle containing 12 ml of horseradish peroxidase labelled monoclonal anti-GSA with antimicrobial agents.

CONTROL +

One dropper bottle containing 4 ml of a buffered solution with inactivated Giardia antigen, and antimicrobial agents

### CONTROL -**Negative Control**

**Positive Control** 

One dropper bottle containing 4 ml of a buffered solution with a red dye, and antimicrobial agents

### SAMPLE DILUENT **Specimen Dilution Buffer**

One bottle containing 120 ml of a buffered solution with rabbit serum, a red dye and

### WASH BUFFER (x10)

antimicrobial agents. Wash Buffer One bottle containing 120 ml of a (x10)

concentrated buffered solution with

antimicrobial agents. Dilute (x10) Wash Buffer concentrate to (x1) by adding 1 part concentrate to 9 parts distilled or deionised water. Diluted Wash Buffer is stable for 1 month when stored at 2 - 8°C.

### SUBSTRATE TMB

### Colour Substrate

One dropper bottle containing 25 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffer.

The Colour Substrate should be stored in and used from the light protected bottle in which it is provided. If an aliquot is removed from

the original bottle for any reason, do not return unused Colour Substrate to the original

### STOP SOLUTION

### **Stop Solution**

One dropper bottle containing 12 ml of 0.46 mol/l Sulphuric acid.

\*Note: Do not interchange reagents between kits with different lot numbers

### PRECAUTIONS

IVD

The reagents are for in vitro diagnostic use only.

For professional use only.

Please refer to the Material Safety Data Sheet (MSDS) and product labelling for information on potentially hazardous components.

### **HEALTH AND SAFETY INFORMATION**

- Reagents are prepared from biological materials and should be handled as potentially infectious material. Discard using appropriate biohazard procedures.
- Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
- Specimens may contain potentially infectious agents and should be handled at Biosafety Level 2 as recommended in the CDC/NIH manual, "Biosafety in Microbiological and Biomedical Laboratories". 5th Edition.
- Wash Buffer contains potential skin sensitiser (< 1% v/v). Avoid skin contact. Wear disposable Vinyl or Nitrile gloves
- Discard used Wash Buffer in appropriate biohazard

# **ANALYTICAL PRECAUTIONS**

- Carefully read and follow all instructions in this Instruction
- Reagents are provided at the necessary working strength with the exception of the Wash Buffer concentrate. Do not dilute reagents, except where instructed.
- 6.8. Do not use reagents beyond the expiration dates. Expiration dates are printed on each reagent label. Use of reagents beyond the expiration date may affect the accuracy of results.
- The following common reagents may be used across the ProSpecT product range: Wash Buffer, Colour Substrate and Stop Solution.
- Microbial contamination of reagents may decrease the accuracy of the assay. Avoid microbial contamination of reagents by using sterile disposable pipettes when removing aliquots from reagent bottles.
- Allow all reagents and specimens to reach room temperature (20 - 25°C) before use.
- Microplate strips must be stored in the resealable foil pouch, with desiccant, to protect microplate wells from
- Stool samples must be thoroughly mixed prior to specimen processing to ensure accurate representation of the specimen. DO NOT CONCENTRATE SPECIMENS
- 6.14. Colour Substrate is sensitive to light exposure. If the 8.11. reagent is exposed to light and develops colour, the reagent must be discarded.
- Persons who are colour blind or visually impaired may not be able to read the test visually and should use spectrophotometric readings to interpret results.
- Add reagents to the test wells in the same order throughout the procedure. To avoid contamination do not touch the fluid in the wells with the bottle tips.
- Time each incubation accurately. Start timing after adding reagent to the last well on each microplate being tested. To ensure accurate timing, process no more than three 96 well plates at one time. Deviation from the established procedure may alter the performance of the assay.
- It is important to hold the dropper bottles vertically and that the drop forms at the tip of the nozzle. If the nozzle becomes wet, a drop of incorrect volume will form around the end and not at the tip; if this occurs dry the nozzle before progressing.

# **COLLECTION OF FAECAL SPECIMENS**

Specimens collected for routine ova and parasite examination can be used for the ProSpecT Giardia EZ Microplate Assay. Stool specimens should be collected in clean, leak-proof plastic

FRESH Untreated stool specimens should be stored at 2 - 8°C and

FROZEN If fresh specimens cannot be tested within 48 hours, they should be frozen at -20 to -70°C.

PRESERVED Stool specimens treated with 10% formalin or 10.2. Interpretation of visual results: SAF fixatives may be refrigerated (2 - 8°C) or stored at room temperature (20 - 25°C) and should be tested within 2 months

CARY BLAIR Stool specimens collected in Cary Blair Transport  $Medium\, should\, be\, refrigerated\, or\, frozen\, and\, tested\, within\, 1\, week$ after collection. Stool specimens that have been concentrated or treated with PVA or MIF fixatives are not suitable for use.

SWAB/DIAPER Stool specimens obtained from rectal swabs and diapers are acceptable for use in the ProSpecT Giardia EZ Microplate Assay. Please note the use of super absorbent diapers

### is not acceptable. TEST PROCEDURE

# REQUIRED MATERIALS PROVIDED

See Kit Contents, section 5

# MATERIALS REQUIRED BUT NOT PROVIDED

Stool specimen collection containers Timer that measures minutes Wash bottle for Wash Buffer

# Distilled or deionised water **OPTIONAL MATERIALS NOT PROVIDED**

Microplate reader capable of reading 450 nm or 450/620

Cotton or rayon tipped applicator sticks Micropipette to deliver volumes to 200 µl Plastic or glass disposable test tubes Vortex mixer with plate adapter or shaker

### Specimen Preparation for Assay:

- Thaw frozen stool specimens. Stool specimens collected in fixatives or transport media should be emulsified thoroughly by vigorously shaking o vortexing to ensure uniform distribution of antigen.
- 2 Label the required number of tubes with patien identification
- 3 Pipette or pour 1 ml of Specimen Dilution Buffer into each tube.
- 4 Rotate a cotton or rayon tipped applicator in the specimen until it is thoroughly coated:

# Unpreserved specimens: use 1 applicator

Preserved or liquid specimens: use 3 separate applicators (either 3 new applicators or the same applicator introduced into the specimen 3 separate

Alternately, 0.2 ml (200 µl) of preserved or liquid specimen may be pipetted into 1 ml of Specimen Dilution Buffer. 5 Rotate applicators in the Specimen Dilution Buffe

- to suspend the faecal material in solution. Then rol each applicator against the side to express as much fluid as possible. Discard applicators appropriately. Prepared specimens may remain in the Specimen Dilution Buffer at room temperature (20 - 25°C) for up to 8 hours or in the refrigerator (2 - 8°C) for up to 48 hours prior to testing
- Open the foil pouch, remove the required number of microplate strips and place into a microplate strip holder. Use one well for the Negative Control and one well for

6 PROCEED TO STEP 8.2

the Positive Control. If using less than 8 wells, break off the required number of wells from a strip and return the unused wells to the foil pouch with desiccant. RESEAL POUCH TIGHTLY TO EXCLUDE MOISTURE AND RETURN TO

- Pipette 2 drops (100  $\mu$ l) of Enzyme Conjugate into each
- Add  $2\ drops$  (100  $\mu l)$  of Negative Control to well A1. Add 2 drops (100 µl) of Positive Control to well B1.
- Pipette 100  $\mu l$  of each diluted specimen into individual wells. Mix the well contents by tapping gently on the side of the microplate well holder.
- Cover microplate and incubate at room temperature (20 - 25°C) for **60 minutes**. Begin timing after the addition
- Shake out or aspirate the contents of the wells. Wash by completely filling each well with diluted Wash Buffer (~350-400 μl/well). Shake out or aspirate all fluid from the wells after each wash. Wash a total of 5 times. After the last wash remove contents and strike plate on clean paper towels or aspirate. Remove as much Wash Buffer as possible but do not allow the wells to dry out at any time.
- Add 4 drops (200 μl) of Colour Substrate to each well.
- Cover microplate and incubate at room temperature (20 - 25°C) for 10 minutes
- Add **1 drop** (50  $\mu$ l) Stop Solution to each well. Gently tap or vortex the wells until the yellow colour is uniform. Read reactions within 10 minutes after adding the Stop
- Read visually or spectrophotometrically at 450 nm (single Sensitivity 96% wavelength) or 450/620 to 650 nm (dual wavelength).

# **QUALITY CONTROL**

Positive and Negative Controls must be included each time the test is performed. The Positive and Negative Controls serve as both reagent and procedural controls. The controls are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cut-off.

The optical density (O.D.) of the Negative Control should be  $\leq$ 0.100 at 450 nm or < 0.070 at 450/620 to 650 nm. The Negative Control should be colourless when read visually. If yellow colour equal to 1+ or greater on the Procedure Card is present in the Negative Control, the test should be repeated with careful attention to the wash procedure.

The O.D. of the Positive Control should be  $\geq$  0.300 at 450 nm or 450/620 to 650 nm, after the O.D. of the Negative Control is subtracted and should be equal to or greater than the 2+ reaction when read visually. If yellow colour less than 2+ on the Procedure Card is present in the Positive Control, call for technical assistance.

# RESULTS

Refer to the enclosed Procedure Card for colour interpretations. 10.1. Read the test results by comparing with the reaction

colours on the Procedure Card. Positive: yellow colour of at least 1+ intensity

Positive: If yellow colour of at least 1+ intensity develops in the test well, the sample contains GSA 65 and the test

Negative: A colourless reaction is a negative result and indicates that no GSA 65 or an undetectable level of GSA 65 is present in the sample tested.

### **SPECTROPHOTOMETRIC**

- Read results at either single (450 nm) or dual (450/620 to 650 nm) wavelength
- 10.4. Read the optical density (O.D.) for the Negative Control.
  - Subtract the O.D. of the Negative Control well from the O.D. readings of the Positive Control well and the test wells before interpreting results.

Note: Readers may be set to blank on the Negative Control well so that the Negative Control well O.D. is automatically subtracted from all of the other readings. If the reader does not have this capability, blank on air and subtract the O.D. of the Negative Control well from the O.D. readings of the Positive Control well and test wells before interpreting

10.6. Read the test results:

**Positive:** O.D. of  $\geq$  0.050 blanked value

(i.e. after the O.D. of the Negative Control is subtracted) Negative: O.D. of < 0.050 blanked value

(i.e. after the O.D. of the Negative Control is subtracted)

10.7. Interpretation of spectrophotometric results:

Positive: If the blanked O.D. reading is equal to or greater than 0.050 in the test well, the sample contains GSA 65 and the test is positive.

Negative: A blanked O.D. reading less than 0.050 is a negative result and indicates that no GSA 65 or an undetectable level of GSA 65 is present in the sample

\*Note: Any wells that are visually clear but give an O.D. reading that is inconsistent with the visual interpretation should be considered a discrepant reading and examined for the presence of bubbles, small particles in the wells, or an opaque film on the bottom of the well. To remove the film, wipe the underside of the wells and read the O.D. again. If the discrepancy between visual and O.D. readings persists, repeat the test.

# PERFORMANCE LIMITATIONS

The validity of results with the ProSpecT Giardia EZ Microplate Assay depends on the control reaction performing as expected. See Quality Control, section 9.

A negative test result does not exclude the possibility of the presence of Giardia, and may occur when the antigen level in the sample is below the detection level of the test. Correlation between the amount of antigen in a sample and clinical presentation has not been established.

As with all IN VITRO diagnostic tests, results should be interpreted by the clinician in conjunction with clinical findings and/or other laboratory results. Proper specimen collection and processing are essential to

achieve optimal performance of the assay. Optimal test results are obtained from specimens tested as soon after collection as possible. See Collection of Faecal Specimens, section 7.

ProSpecT Giardia EZ Microplate Assay has been classified as high

# **EXPECTED VALUES**

The prevalence of *Giardia* infection varies in different populations and geographic areas. In the U.S., the incidence of Giardia is approximately 4-7% with higher prevalence rates in children<sup>12</sup> and in homosexual males5,6

# PERFORMANCE CHARACTERISTICS

results of these evaluations are presented below

# SENSITIVITY AND SPECIFICITY

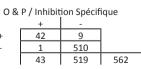
Clinical studies were conducted to evaluate the performance of the ProSpecT Giardia EZ Microplate Assay. Specimens were obtained from a large reference laboratory which performed O&P testing. A total of 254 unpreserved specimens were tested; 100 were positive for Giardia by O&P and 154 were negative. Fiftyfour of the Giardia negative specimens contained parasites other than Giardia by O&P. There were 10 specimens that were O&P negative/EIA positive. Seven of these were reproducibly positive for GSA 65 and specific inhibition tests with antibody to GSA 65 showed greater than 50% inhibition of the reaction with all 7 specimens. When these are considered to be true positives, the

# O & P / Specific Inhibition

Specificity 98%

ProSpecT

A trial was conducted with 562 prospectively collected specimens tested by O&P. The specimens were collected from a large metropolitan hospital reference laboratory (360 unpreserved specimens) and from a public health laboratory (202 specimens preserved in formalin). There was one O&P positive/ EIA negative result and 10 specimens were O&P negative/EIA positive. One of these was GSA 65 positive by specific inhibition. The performance of the ProSpecT Giardia EZ Microplate Assay in this trial is given



Sensitivity 98% Specificity 98%

# ANALYTICAL SENSITIVITY

The ProSpecT Giardia EZ Microplate Assay detects approximately 15.6 nanograms/ml of GSA 65.

# REPRODUCIBILITY

The inter-assay or run-to-run coefficient of variation (CV) of the ProSpecT Giardia EZ Microplate Assay was evaluated by selecting four positive specimens with varying optical density readings. Each sample was tested in 10 wells per day for five consecutive days. The mean inter-assay CV was 2.7%.

Sample	Mean O.D.	Standard Deviation	%CV	
1	1.40	0.014	1.0	
2	0.78	0.038	3.8	
3	0.26	0.008	3.1	
4	0.12	0.004	2.9	

The intra-assay or within-run CV was evaluated by testing 24 wells with each of 4 positive specimens. The mean intra-assay CV was 4.23%.

Sample	Mean O.D.	Standard Deviation	%CV
1	1.62	0.047	2.9
2	0.91	0.053	5.8
3	0.53	0.019	3.5
4	0.38	0.018	4.7

### CROSS-REACTIVITY

The ProSpecT Giardia EZ Microplate Assay has been tested with stool specimens found to be O&P positive for a number of faecal parasites. No cross reactivity was observed with any of the infectious agents listed below.

Ascaris lumbricoides Entamoeba histolytica Blastocystis hominis Enterobius vermicularis Chilomastix mesnili Hookworm Cryptosporidium parvum Hymenolepis nana Dientamoeba fragilis Iodamoeba butschlii Strongyloides stercoralis Endolimax nana

Entamoeba hartmanni

Entamoeba coli

### **BIBLIOGRAPHY**

### 1. Addiss, D.G., 1991.

 $\label{lem:eq:commercially} \textbf{Evaluation of a commercially available Enzyme-Linked Immunoabsorbent}$ Assay for Giardia lamblia Antigen in Stool. J. Clin. Microbiol. 29(6):1137-1142.

Trichuris trichiura

2. Black, R.E. et al., 1977.

Giardiasis in day care centres: evidence of person to person transmission.

Paediatrics 60(4):486-491.

3. Centre for Disease Control, Atlanta, GA, 1978.

Intestinal parasite surveillance - United States, 1976.

Morbid. Mortal. Weekly Rep. 27(20):167-168.

Craun, G.F., 1979.

Waterborne Giardiasis in the United States: A Review. Am. J. Public Health. 69(8):817-819.

5. Craun, G.F., 1986.

Waterborne Giardiasis in the United States 1965-84.

Lancet I:513-514.

6. Kean, B.H. et al., 1979.

Epidemic of amoebiasis and giardiasis in a biased population.

Br. J. Ven. Dis. 55:375-378.

Meyers, J.D. et al. 1977.

Giardia lamblia infection in homosexual men.

Br. J. Ven. Dis. 53:54-55.

8. Rendtorff, R.C., 1979. The experimental transmission of  $\emph{Giardia lamblia}$  among volunteer

subjects in waterborne transmission of Giardiasis. (Jakubowski, W., and J.C. Hoff, eds.), pp. 64-8. EPA, U.S., Cincinnati, OH.

# 9. Rosoff, J.D., and H.H. Stibbs. 1986.

Isolation and identification of a Giardia lamblia-Specific Stool Antigen (GSA 65) Useful in Coprodiagnosis of Giardiasis.

### J. Clin. Microbiol. 23(5):905-910. 10. Rosoff, J.D., and H.H. Stibbs., 1986.

Physical and chemical characterization of a Giardia lamblia-Specific Antigen useful in the coprodiagnosis of Giardiasis.

J. Clin. Microbiol. 24(6):1079-1083. 11. Rosoff, J.D. et al., 1989.

Stool diagnosis of Giardiasis using a commercially available enzyme immunoassay to detect Giardia-Specific Antigen 65 (GSA 65). J. Clin. Microbiol. 27(9):1997-2002.

# 12. Schieven, B.C. and Z. Hussain., 1990.

Evaluation of an enzyme immunoassay test kit for diagnosing infections with Giardia lamblia.

Serodiag and Immunother. 4:109-113.

# 13. Sonnad, S., L. Bahrami, P. O'Hanley, 1991.

 ${\bf Compatibility\ Assessment\ of\ Three\ Common\ Transport\ Media\ Systems}$ with the  $\mathsf{ProSpecT^{\mathsf{TM}}}$  /  $\mathit{Giardia}$  Immunoassay. Presented at the 1991 American Society for Microbiology Meeting,

Session 20. 14. Tijssen, P.,

 $\label{practice} \mbox{Practice and Theory of Enzyme Immunoassays.}$ 

Laboratory Techniques in Biochemistry and Molecular Biology, R.H. Burdon and P.H. van Knippenberg, eds., Elsevier, N.Y., 1985, pp.14-16.

### 15. Visvesvara, G.S., 1982. Giardiasis in Children.

J. Pediatric Gastroentrol Nutr. 1(4):463-465.

# 16. Wolfe, M.S., 1979.

Managing the patient with giardiasis: clinical, diagnostic and therapeutic

Waterborne Transmission of Giardiasis. (W. Jakubowski, and J.C. Hoff, eds.), pp.39-52. EPA, U.S., Cincinnati, OH.

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Oxoid Ltd Wade Road Basingstoke Hants, RG24 8PW

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