RML # 2458024
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Alexon·Trend

Advancing
the Diagnosis
of Intestinal Infections

An Enzyme Immunoassay for the Detection of *Giardia* Specific Antigen

ProSpecT®
Giardia Microplate Assay

PACKAGE INSERT

Remel # 2458024, 24
" 8096, 96

For *IN VITRO* Diagnostic Use

24/96 Tests

Catalog #580-24/580-96M

U.S. Patent No. 5,503,983

Alexon-Trend, Inc., 14000 Unity St. NW, Ramsey, MN 55303

Made in the USA
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ProSpecT® *Giardia* Microplate Assay

INTENDED USE

ProSpecT® *Giardia* Microplate Assay uses monoclonal antibody for the qualitative detection of *Giardia* Specific Antigen (GSA 65) in aqueous extracts of fecal specimens.

SUMMARY AND EXPLANATION OF THE TEST

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 (3). This parasite has been implicated in a number of epidemics (4,5) 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 (1, 15) and in homosexual males (6, 7). Acute symptoms of giardiasis may include diarrhea, malabsorption, abdominal cramps, anorexia, nausea, weight loss, flatulence, anemia, and general weakness lasting from several weeks to several months (16). 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 (2).

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 (9,10,11,12). 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 cysts or trophozoites. (9,10,11). 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 (1,11,12,13).

PRINCIPLES OF THE PROCEDURE

ProSpecT® *Giardia* Microplate Assay is a solid phase immunoassay for the detection of GSA 65 (14). Diluted stool specimens are added to break-away microplate wells on which anti-GSA 65 antibody is bound. If GSA 65 is present, it is 'captured' by the bound antibody. The wells are incubated and then washed to remove unbound material. The enzyme conjugate (monoclonal anti-GSA antibody labeled with horseradish peroxidase enzyme) is added. The wells are incubated and then washed to remove unbound enzyme conjugate. In a positive reaction, GSA 65 binds the enzyme conjugate to the well. The substrate for the enzyme, TMB, is added. In a positive reaction, the enzyme bound to the well by GSA 65 converts the substrate to a colored reaction product. Color 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 colored reaction product develops.

REAGENTS

The ProSpecT® *Giardia* Microplate Assay includes sufficient reagents to perform 24 or 96 tests. The kit also includes 24 or 96 transfer pipets.

Reagents:	24 Test/96 Test
Microplate Coated with rabbit anti-GSA 65 antibody	8 wells per strip 3 strips/12 strips
Enzyme Conjugate Peroxidase labeled mouse monoclonal anti-GSA with bovine serum and 0.01% thimerosal	1 bottle 5 ml/25 ml
Positive Control Human fecal material with 0.02% thimerosal	1bottle 4 ml
Negative Control Human fecal material with 0.02% thimerosal	1 bottle 4 ml
Specimen Dilution Buffer Buffered solution with rabbit serum and 0.02% thimerosal	1 bottle 35 ml/110 ml
Wash Buffer 10x concentrated buffered solution with 0.1% thimerosal	1 bottle 50 ml/110 ml
Color Substrate TMB in buffer	1 bottle 5 ml/25 ml
Stop Solution 0.5 N Hydrochloric acid (corrosive)	1bottle 6 ml

WARNINGS AND PRECAUTIONS

The ProSpecT® *Giardia* Microplate Assay is intended for in vitro diagnostic use by professionals only. The assay is a qualitative assay for the detection of *Giardia lamblia* Specific Antigen. The intensity of color development does not correlate with the level of antigen or degree of infection.

Specimens, specimen dilutions, Positive Controls, Negative Controls and microplate test strips should be handled using standard guidelines for biohazard materials. Proper handling and disposal methods should be established by the laboratory.

Wash Buffer, Enzyme Conjugate, Specimen Dilution Buffer and Positive and Negative Controls contain thimerosal which may be irritating to skin, eyes and mucous membranes. In case of contact, flush eyes or skin with copious amounts of water.

Stop Solution is corrosive and should be handled with care. If this solution comes into contact with skin or clothing, it should be washed off immediately.

Color Substrate is sensitive to light exposure. If the reagent is exposed to light and develops color, the reagent must be discarded.

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.

DO NOT CONCENTRATE SPECIMENS BEFORE TESTING.

PREPARATION OF REAGENTS

Before use, bring all reagents to room temperature (20 - 25°C) and mix gently. Return the unused reagents to the refrigerator after use.

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

Dilute 10x Wash Buffer concentrate to 1x by adding 1 part concentrate to 9 parts distilled or deionized water. Diluted Wash Buffer is stable for 1 month when stored at 2 - 8°C.

STORAGE AND HANDLING

The expiration date of each kit is stated on the package label. Store all components at 2 - 8°C. Unused microplate strips should be stored in the foil pouch containing desiccant to exclude moisture. The Color 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 Color Substrate to the original bottle.

COLLECTION OF STOOL SPECIMENS

Specimens collected for routine ova and parasite examination can be used for the ProSpecT® *Giardia* Microplate Assay. Stool specimens should be collected in clean, leak-proof plastic containers. Fresh, untreated stool specimens should be stored at 2 - 8°C and tested within 48 hours. If fresh specimens cannot be tested within 48 hours, they should be frozen at -20 to -70°C. Stool specimens treated with 10% formalin, MF or SAF fixatives may be refrigerated (2 - 8°C) or stored at room temperature (20 - 25°C) and should be tested within 2 months after collection. Stool specimens collected in C&S Transport Medium (or equivalent) should be refrigerated or frozen and tested within 1 week after collection. Stool specimens that have been concentrated or treated with PVA fixatives are not suitable for use. Stool specimens obtained from rectal swabs and diapers are acceptable for use in the ProSpecT® *Giardia* Microplate Assay.

PROCEDURE NOTES

- Carefully read and follow all instructions in this package insert.
- Allow all reagents and specimens to reach room temperature (20 - 25°C).
- 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 waiting period 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.

TEST PROCEDURE

Required materials provided:
Reagents
Microplate Stripwell Holder

Required materials not provided:
Stool specimen collection containers
Wash bottle or dispenser for Wash Buffer
Timer that measures minutes
Distilled or deionized water

Optional materials not provided:
Microplate reader (spectrophotometer)
Cotton or rayon tipped applicator sticks
Micropipet to deliver volumes to 200µl
Plastic or glass disposable test tubes
Vortex mixer with plate adapter or shaker

Procedure:

1. 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 the Positive Control. If using less than 8 wells, break off the required number of wells from a strip and return the rest to the foil pouch. RESEAL POUCH TIGHTLY TO EXCLUDE MOISTURE AND RETURN TO THE REFRIGERATOR.
2. Specimens can be added directly into the wells or pre-diluted in tubes before adding to the wells. Pre-diluted specimens can be held at room temperature (20 - 25°C) for 8 hours or at 2-8°C for 48 hours prior to testing (see below). Choose one of these two methods: See Box A for dilution in wells; See Box B for pre-dilution in tubes.

A Dilution in Wells

3. Unpreserved Solid Specimens: Label one tube for each specimen. Add 0.4 ml Specimen Dilution Buffer (SDB) to each tube. Coat 1 swab with specimen and vigorously mix into SDB. Express as much fluid as possible and discard the swab. Put a transfer pipet into the tube.
4. Preserved or Watery Unpreserved Specimens: Mix by shaking specimen collection containers. No further preparation is necessary.
5. Add 4 drops Negative Control to well A1. Add 4 drops Positive Control to well B1.
6. Add 100 µl SDB to each specimen well.
7. Using transfer pipets add 1 drop of each specimen to a well.

   Note: Place the opening of the transfer pipets just inside the wells to avoid splashing into adjacent wells.

   PROCEED TO STEP 8

B Dilution in Tubes

3. Unpreserved Solid Specimens: Label one tube for each specimen. Add 1 ml Specimen Dilution Buffer (SDB) to each tube. Coat 1 swab with specimen and vigorously stir into SDB. Express as much fluid as possible and discard the swab. Put a transfer pipet into each tube.
4. Preserved or Watery Unpreserved Specimens: Label one tube for each specimen. Add 1 ml SDB to each tube. Mix samples by shaking specimen collection containers. Using transfer pipets draw up 0.3 ml (third mark from the tip of the pipet). Expel sample into SDB. Mix by drawing up and down once. Leave transfer pipets in the tubes.

   Diluted specimens may be held for 8 hours at room temperature (20 - 25°C) or 48 hours at 2-8°C.
5. Add 4 drops Negative Control to well A1.
6. Add 4 drops Positive Control to well B1.
7. Using transfer pipets add 0.2 ml (second mark from the tip of the pipet) of each specimen to a well.

   Note: Place the opening of the transfer pipets just inside the wells to avoid splashing into adjacent wells.

   PROCEED TO STEP 8

8. Incubate the microplate at room temperature (20 - 25°C) for **60 minutes.** Begin timing after the addition of the last specimen.
9. 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 wells after each wash. Wash a total of 3 times. After the last wash dump out contents and bang 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.
10. Add 4 drops (200 µl) of Enzyme Conjugate (blue cap) to each well.
11. Incubate the microplate at room temperature (20 - 25°C) for **30 minutes.**
12. Decant and wash each well 5 times as in step 9.
13. Add 4 drops (200 µl) of Color Substrate to each well.
14. Incubate the microplate at room temperature (20 - 25°C) for **10 minutes.**
15. Add 1 drop (50 µl) of Stop Solution to each well. Gently tap or vortex the wells until the yellow color is uniform. Read reactions within 10 minutes after adding Stop Solution. Read visually or spectrophotometrically at 450 nm.

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.

RESULTS

Refer to the enclosed Procedure Card for color interpretations.

Visual:

1. Read the Negative Control. The reaction should be colorless. If yellow color equal to 1+ or greater is present, the test should be repeated with careful attention to the wash procedure. Call Alexon for technical assistance.
2. Read the Positive Control. The intensity of color in the Positive Control should be equal to or greater than the 2+ reaction on the procedure card. If there is less color, call Alexon for technical assistance.
3. Read the test results by comparing with the reaction colors on the procedure card.

   Positive: yellow color of at least 1+ intensity
   Negative: colorless
4. Interpretation of visual results:

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

   Note: Tests with faint yellow color (less than 1+) should be repeated.

   Negative: A colorless 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:

1. Set the spectrophotometer (microplate reader) to read at 450 nm.
2. Read the optical density (O.D.) for the Negative Control. For a valid test, the O.D. of the Negative Control should be 0.100 or less. If the O.D. is greater than 0.100, the results are invalid and the test should be repeated with careful attention to the wash procedure. Call Alexon for technical assistance.
3. 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 results.
4. The O.D. for the Positive Control should be 0.300 or greater after the O.D. of the blank is subtracted. If the O.D. is less than 0.300, the test should be repeated. Call Alexon for technical assistance.

5. Read the test results:

   Positive: O.D. of ≥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)

6. 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 tested.

Limitations of the Procedure

A single diagnostic assay or test result should not be used as the only basis for forming a clinical conclusion. Results should be supported by correlation with patient symptoms and the overall clinical picture.

ProSpecT® *Giardia* Microplate Assay has been classified high complexity and assigned analyte identifier code 2222 and test system codes 04557 (spectro) and 04558 (visual).

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 (12) and in homosexual males (5,6).

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

Clinical studies were conducted to evaluate the performance of the ProSpecT® *Giardia* Microplate Assay. Specimens were obtained from a large reference laboratory which performed O&P testing. A total of 248 unpreserved specimens were tested; 101 were positive for *Giardia* by O&P and 147 were negative. Forty-seven of the *Giardia* negative specimens contained parasites other than *Giardia* by O&P. All of the O&P positive specimens were positive in the microplate assay and all of the negative specimens were negative. The performance of the ProSpecT® *Giardia* Microplate Assay in this study is presented below.

		O&P +	O&P −	
ProSpecT *Giardia*	+	101	0	
	−	0	147	
		101	147	248

Sensitivity 101/101 = 100% (96-100%)
Specificity 147/147 = 100% (98-100%)

Numbers in parentheses are 95% confidence intervals.

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* Microplate Assay in this trial is given below.

		O&P/Specific Inhibition +	O&P/Specific Inhibition −	
ProSpecT *Giardia*	+	42	9	
	−	1	510	
		43	519	562

Sensitivity 42/43 = 98% (88-100%)
Specificity 510/519 = 98% (97-99%)

Numbers in parentheses are 95% confidence intervals.

Analytical Sensitivity

The ProSpecT® *Giardia* Microplate Assay detects approximately 3.9 nanograms/ml of GSA 65.

Reproducibility

The inter-assay or run-to-run coefficient of variation (CV) was evaluated with 5 positive and 5 negative samples assayed at least ten times in three separate runs. For the dilution in tube procedure the mean CV of the negative samples was 3.02% (range 0.93% to 3.90%) and the mean CV for the positive samples was 4.65% (range 1.74% to 10.72%). For the dilution in wells procedure the mean CV of the negative samples was 7.66% (range 4.22% to 16.19%) and the mean CV for the positive samples was 7.76% (range 4.12% to 11.26%).

The intra-assay or within-run CV was evaluated with 5 positive and 5 negative samples assayed at least ten times in a single run. For the dilution in tube procedure the mean CV of the negative samples was 4.47% (range 3.30% to 5.39%) and the mean CV for the positive samples was 5.61% (range 2.53% to 10.12%). For the dilution in wells procedure the mean CV of the negative samples was 4.51% (range 2.73% to 5.36%) and the mean CV for the positive samples was 9.58% (range 4.78% to 13.8%).

Cross-Reactivity

The ProSpecT® *Giardia* Microplate Assay has been tested with stool specimens found to be positive for a number of fecal organisms. No cross-reactivity was observed with any of the infectious agents listed below.

Ascaris lumbricoides (5)
Blastocystis hominis (8)
Cryptosporidium parvum (10)
Dientamoeba fragilis (10)
Entamoeba coli (13)
Entamoeba hartmanni (5)
Entamoeba histolytica (5)
Endolimax nana (6)
Hymenolepis nana (2)
Iodamoeba butschlii (9)
Isospora belli (5)
Rotavirus (11)
Strongyloides stercoralis (1)
Trichuris trichiuris (2)

Numbers in parentheses indicate the numbers of specimens tested.

ORDERING

ProSpecT® *Giardia* Microplate Assay catalog number is 580-96M (96 test) and 580-48 (48 test). For ordering or customer and technical assistance please call 763-323-7800 or **800-366-0096** or FAX 763-712-2371.

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