**Giardia Microplate Assay**

**Procedure Card**

**All reagents, except the Wash Buffer, are supplied at working concentration.** See also Kit Contents, preparation for use and storage. See also: 5 KIT CONTENTS, PREPARATION FOR USE AND STORAGE.

**PRINCIPLE OF THE TEST**

*ProSpecT* Giardia Microplate Assay is a solid-phase immunoassay for the detection of Giardia antigen in faecal specimens. The antigen is captured by a specific monoclonal antibody labeled with alkaline phosphatase and conjugated to a red and antimonial antigen. The alkaline phosphatase activity is measured spectrophotometrically. The test is performed using fresh faecal specimens from which no cysts or trophozoites can be seen by examination with a routine light microscope. Giardia is considered to be a frequent cause of human diarrhoea and has been found in 1-2% of the world’s population, with 10% prevalence in school children and children under 5 years of age. The incidence of the disease varies considerably throughout the world. In the U.S., the incidence of giardiasis is approximately 4-7% with higher prevalence rates in children and symptomatic patients. In the U.S., the incidence of giardiasis is approximately 4-7% with higher prevalence rates in children and symptomatic patients.

**PRECAUTIONS**

1. **Temperature Limitation** (Storage Temp.)
   - In Vitro Diagnostic Medical Device
   - Do not use reagents beyond the expiration dates.
   - Do not interchange reagents between kits with different expiration dates.
   - Preserved and Unprotected Specimens: Mix the test specimen with the wash buffer immediately before using it.

2. **Color Substrate**
   - Microplate reader capable of reading 450 nm or 450/650 nm (dual wavelength) for absorbance measurement.

3. **Wash Buffer**
   - Microplate reader capable of reading 450 nm or 450/650 nm (dual wavelength) for absorbance measurement.

4. **Procedure**
   - SPECIMEN COLLECTION: Specimens collected for routine ova and parasite examination
     - Stool specimens obtained from rectal swabs and CARY BLAIR or SAF fixatives may be refrigerated (2 - 8°C) or stored at room temperature (14 - 24°C) for up to 7 days. If fresh specimens cannot be tested within 48 hours, they should be frozen at -20 to -70°C.
     - If fresh specimens cannot be tested within 48 hours, they should be frozen at -20 to -70°C.

5. **Materials Required**
   - Microplate reader capable of reading 450 nm or 450/650 nm (dual wavelength) for absorbance measurement.

6. **Interpretation of results**
   - Positive: If yellow colour of at least 1+ intensity develops in the test well after 10 minutes, the sample contains GSA 65 and the test is positive.
   - 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.

7. **Sensitivity and Specificity**
   - Sensitivity: 100% (95% CI = 93 - 100)
   - Specificity: 99.0% (95% CI = 98 - 100)

**1 INTENDED USE**

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

**2 INDICATIONS**

*ProSpecT* Giardia Microplate Assay is now recognized as an important human intestinal disease in most areas of the world. The causal organism, *Giardia lamblia*, is the most frequently identified protozoan of all species. The parasite has multiplies in numbers of species[1] and the endemicity in the U.S. is well-recognized. Proportion in adults is estimated at 4 - 7%. Higher prevalence rates are seen in children[1] and in homosexual males[2]. Acute symptoms of giardiasis may include diarrhea, malabsorption, anorexia, malaise, weight loss, flatulence, and anaemia, and general weakness lasting from several weeks to several months[3]. Chronic infections can also occur with or without an acute phase, are often associated with treatment failure, and may result in severe anaemia and symptoms. Infection with *Giardia* may also be asymptomatic.[4]

*Giardia* Spontaneous (GSA 65) is a macromolecule that has been used in *ProSpecT* Giardia Microplate Assay and has been used as the basis of immunological[5-7] and ELISA[8]. *ProSpecT* Giardia Microplate Assay contains Giardia antigen in faecal specimens with visible signs of cysts or trophozoites. *ProSpecT* Giardia Microplate Assay is intended for the detection of Giardia antigen in faecal specimens with visible signs of cysts or trophozoites. *ProSpecT* Giardia Microplate Assay is intended for the detection of Giardia antigen in faecal specimens with visible signs of cysts or trophozoites.

**3 DETAILED INSTRUCTIONS**

**PROCEDURE**

8.1 Open the foil pouch, remove the required number of specimens, and place them in the wells of the microplate. Use one well for the Negative Control and one well for the Positive Control. If using less than 8 wells, break off the second strip of foil when 8 wells are complete and then add unused wells to the well with fleece with wash buffer. REPROBE Plate: SDB. MIXING PROTOCOL: PROCEDURE.

8.2 Specimen collection containers. The validity of results with the *ProSpecT* Giardia Microplate Assay depends on the control reaction performing as expected. See also: 5.1 SPECIMEN COLLECTION.

8.3 Use one drop of specimen per well. **Note:** Do not use reagents beyond the expiration dates.

8.4 Shake out or aspirate the contents of the wells. Wash each well with 250 µl of Wash Buffer. Begin timing after the addition of the Wash Buffer. **Note:** Do not use reagents beyond the expiration dates.

8.5 Dilute (x10) Wash Buffer concentrate into wash buffer. **Note:** Do not use reagents beyond the expiration dates.

8.6 Colour Substrate
   - Microplate reader capable of reading 450 nm or 450/650 nm (dual wavelength) for absorbance measurement.

8.7 Shake out or aspirate and wash each well with 250 µl of Wash Buffer. Begin timing after the addition of the Wash Buffer. **Note:** Do not use reagents beyond the expiration dates.

8.8 Do not interchange reagents between kits with different expiration dates. **Note:** Do not use reagents beyond the expiration dates.

8.9 Add the diluted specimen to the wells. **Note:** Do not use reagents beyond the expiration dates. **Note:** Do not use reagents beyond the expiration dates.

8.10 Add 300 µl to each well
   - Microplate reader capable of reading 450 nm or 450/650 nm (dual wavelength) for absorbance measurement.

8.11 Read visually or spectrophotometrically at 450 nm (single wavelength) or 450/620 to 650 nm (dual wavelength).

9.1 Interpretation of visual results: 
   - Positive: If yellow colour of at least 1+ intensity develops in the test well after 10 minutes, the sample contains GSA 65 and the test is positive.
   - 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.

**10 PERFORMANCE LIMITATIONS**

Perform the test only with a single (450 nm) or dual (450/650 nm) microplate reader.

**11 EXPECTED VALUES**

The prevalence of Giardia infection varies in different populations and geographic areas. In the U.S., the incidence of Giardiasis is approximately 4-7% with higher prevalence rates in children and in homosexual males[2].

**12 PERFORMANCE CHARACTERISTICS**

**Sensitivity and Specificity**

Clinical studies were conducted to evaluate the performance of the *ProSpecT* Giardia Microplate Assay compared to ELISA. A large number of samples were tested from a large reference laboratory which performed O&P testing and *ProSpecT* Giardia Microplate Assay. The samples were selected to be representative of the world population and the results were analyzed using a two-by-two table. All enrolled samples were positive for GSB by O&P and 147 were negative. Forty-seven of the *ProSpecT* Giardia Negative specimens were positive for the *ProSpecT* Giardia Negative specimens in the microplate assay and all of the negative specimens were negative. The overall sensitivity of the *ProSpecT* Giardia Microplate Assay in this study was presented by G & P.

**9 QUALITY CONTROL**

Positive and Negative Controls must be included in each test. The Positive and Negative Controls serve as both an internal control for each assay and a control to monitor for substantial reagent failure. The Positive Control will serve as an additional control to monitor for substantial reagent failure. The optical density (O.D.) of the Negative Control should be < 0.050 blanked value. The Positive Control should be retested with care. The optical density (O.D.) of the Negative Control should be ≤ 0.050 blanked value. The Positive Control should be retested with care.
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/LiA negative result and 10 specimens were O&P negative/LiA positive. One of these was GSA 65 positive by specific inhibition. The performance of the ProSpecT Giardia Microplate Assay in this trial is given in the following chart:

<table>
<thead>
<tr>
<th>ProSpecT</th>
<th>Specific Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. lamblia</td>
<td>+</td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td>+</td>
</tr>
<tr>
<td>Strongyloides stercoralis</td>
<td>+</td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td>+</td>
</tr>
<tr>
<td>Entamoeba hartmanni</td>
<td>+</td>
</tr>
<tr>
<td>Isospora belli</td>
<td>+</td>
</tr>
</tbody>
</table>

Sensitivity: 42/43 = 98% (97 - 99%)
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 0.91% to 3.90%) and the mean CV for the positive samples was 4.55% (range 3.74% to 10.72%). For the dilution in wells procedure the mean CV of the negative samples was 7.86% (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 positive samples was 4.47% (range 3.30% to 5.39%) and the mean CV for the positive samples was 5.61% (range 2.5% 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 5.58% (range 4.78% to 13.8%).

**CROSS-REACTIVITY**

No cross-reactivity was observed with any of the infectious agents listed below.

- *Entamoeba intestinalis* (1)
- *Microsporidia Komoki* (6)
- *Cryptosporidium parvum* (10)
- *Dientamoeba fragilis* (10)
- *Blastocystis hominis* (10)
- *Strongyloides stercoralis* (10)
- *Trichuris trichiura* (10)

Numbers in parentheses indicate the number of specimens tested.

**BIBLIOGRAPHY**


ProSpecT™ is a registered trademark.