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ProSpecT™ Entamoeba histolytica Microplate Assay

REF R2456048 .....48 Tests
REF R2456096 .....96 Tests

1 INTENDED USE

ProSpecT Entamoeba histolytica Microplate Assay is for the qualitative detection of *E. histolytica* Specific Antigens (EHSA) in aqueous extracts of human faecal specimens. The assay detects EHSA from both pathogenic and nonpathogenic strains of the organism.

2 SUMMARY

Entamoeba histolytica is an intestinal amoeba which causes infection after ingestion of cysts. Approximately 12% of the world population is infected with *E. histolytica*[1,7]. Ninety percent of infected individuals experience asymptomatic infections and constitute a vast reservoir of the organism. About 10% of infected individuals exhibit clinical symptoms ranging from nonspecific symptoms of gastrointestinal disease to dysentery, colitis, and amoebiasis. Of these, an estimated 2-20% will progress with extra-intestinal invasion and the formation of abscesses, especially in the liver[1,7].

Diagnosis of amoebiasis has traditionally been by ova and parasite (O&P) microscopic observation of the organism in faecal or tissue samples. Microscopy is complicated by the fragile nature of the trophozoites and the intermittent shedding of the life forms. It has been shown that even with 6-9 O&P examinations, diagnosis was made only 72-76% of the time[4]. In one study, the probability of diagnosis based upon one O&P was shown to be only 50% when 100,000 cysts were excreted daily or 0.45% when 1000 cysts were excreted[3]. Significant problems caused by misdiagnosis of *E. histolytica* have been documented[2]. False negative results occur when the parasite is missed by inexperienced technicians and false positive results occur when leukocytes, other amoebae, blood cells or debris are misidentified as *E. histolytica*[2].

In extra-intestinal amoebiasis, organisms may not be excreted into the stool and serologic tests may be necessary for diagnosis. However, problems with serologic tests occur when antibody titres are low or difficult to interpret. In endemic areas a high prevalence of seropositivity complicates diagnosis of current infection. Asymptomatic cyst passers may have negative serologic results[1]. Antibody persistence for prolonged periods after infection complicates the distinction between current and past infection.

Multiplication of *E. histolytica* in the intestinal tract results in the production of specific antigens (EHSA) which are present in the faeces of infected persons. EHSA can be detected with a single stool specimen compared with multiple stool specimens required for O&P. Detection of EHSA eliminates common diagnostic problems such as misidentification, failure to correctly identify the organisms and persistent or absent antibody titres. ProSpecT Entamoeba histolytica Microplate Assay is an assay for detection of EHSA.

3 PRINCIPLES OF THE TEST

ProSpecT Entamoeba histolytica Microplate Assay is a solid phase immunoassay for the detection of EHSA[6].

Diluted stool specimens are added to break-away microplate wells on which anti-EHSA antibodies are bound. If EHSA is present, it is 'captured' by the bound antibody. The wells are incubated and then washed to remove unbound material. The enzyme conjugate (anti-EHSA antibody labelled with horseradish peroxidase enzyme) is added. The wells are incubated and then washed to remove unbound enzyme conjugate. In a positive reaction, EHSA binds the enzyme conjugate to the well. The substrate for the enzyme, 3,3',5,5'-tetramethylbenzidine (TMB), is added and a coloured reaction product is formed. Colour development can be detected visually or spectrophotometrically. In a negative reaction, there is no EHSA or an insufficient level of EHSA present to bind the enzyme conjugate and no coloured reaction product develops.

4 SYMBOL DEFINITIONS

REF	Catalogue Number
IVD	In Vitro Diagnostic Medical Device
Σ	Contains sufficient for <n> tests
	Consult Instructions for Use (IFU)
8°C 2°C	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufacturer
DILUTED SAMPLE	Diluted Sample

5 KIT CONTENTS, PREPARATION FOR USE AND STORAGE

The ProSpecT Entamoeba histolytica Microplate Assay includes sufficient reagents to perform Σ 48 or Σ 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 after use.

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) 6 strips (R2456048) or 12 strips (R2456096) coated with rabbit anti-EHSA antibodies. Unused microplate strips should be stored in the foil pouch containing desiccant to exclude moisture.
CONJUGATE	**Enzyme Conjugate*** One dropper bottle containing 12 ml (R2456048) or 25 ml (R2456096) of horseradish peroxidase-labelled rabbit anti-EHSA in a protein matrix containing bovine serum albumin with antimicrobial agents.
CONTROL +	**Positive Control** One dropper bottle containing 4 ml of purified EHSA in a protein matrix with antimicrobial agents.
CONTROL –	**Negative Control** One dropper bottle containing 4 ml of a protein matrix, 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 antimicrobial agents.
WASH BUFFER (x10)	**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 12 ml (R2456048) or 25 ml (R2456096) 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 bottle.
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.

6 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

6.1 Reagents are prepared from biological materials and should be handled as potentially infectious material. Discard using appropriate biohazard procedures.

6.2 Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.

6.3 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.

6.4 Wash Buffer contains potential skin sensitiser (< 1% v/v). Avoid skin contact. Wear disposable Vinyl or Nitrile gloves.

6.5 Discard used Wash Buffer in appropriate biohazard containers.

ANALYTICAL PRECAUTIONS

6.6 Carefully read and follow all instructions in this Instruction for Use.

6.7 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.

6.9 The following common reagents may be used across the ProSpecT product range: Wash Buffer, Colour Substrate and Stop Solution.

6.10 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.

6.11 Allow all reagents and specimens to reach room temperature (20 - 25°C) before use.

6.12 Microplate strips must be stored in the resealable foil pouch, with desiccant, to protect microplate wells from moisture.

6.13 Stool samples must be thoroughly mixed prior to specimen processing to ensure accurate representation of the specimen. DO NOT CONCENTRATE SPECIMENS BEFORE TESTING.

6.14 Colour Substrate is sensitive to light exposure. If the reagent is exposed to light and develops colour, the reagent must be discarded.

6.15 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.

6.16 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.

6.17 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.

6.18 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.

7 COLLECTION OF FAECAL SPECIMENS

Stool specimens that have been concentrated or collected in 10% formalin, SAF or PVA fixatives are not suitable for use.

FRESH Unpreserved stool specimens should be collected in clean, leak-proof plastic containers. Specimens should be stored at 2 - 8°C and tested within 48 hours.

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

CARY BLAIR Stool specimens collected in Cary Blair Transport Medium should be refrigerated at 2 - 8°C and tested within 1 week after collection.

8 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 to 650 nm
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

PROCEDURE

8.1 **Specimen Preparation for Assay:**
Prepare 1:10 v/v dilutions of each stool specimen.

1. Thaw frozen stool specimens. Stool specimens should be emulsified thoroughly by vigorously shaking or vortexing to ensure uniform distribution of antigen.
2. Label the required number of tubes with patient identification.
3. Pipette or pour **1 ml** of Specimen Dilution Buffer into each tube.
4. With solid or semi-solid specimens, use **one swab** thoroughly coated with faecal material. With liquid specimens, use **3 swabs or 300 µl**.
5. Rotate the applicator(s) several times in the Specimen Dilution Buffer to suspend the faecal material in solution. Then roll the applicator(s) firmly against the side of the vial 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.

8.2 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 unused wells to the foil pouch, with desiccant. RESEAL POUCH TIGHTLY TO EXCLUDE MOISTURE AND RETURN TO THE REFRIGERATOR.

8.3 Add **4 drops** (200 µl) Negative Control, Positive Control, or diluted patient specimen into individual wells taking care not to contaminate surrounding wells. The controls may be dispensed directly from the dropper bottle into the appropriate well or, if desired, 200 µl may be pipetted out of the bottle.

8.4 **Cover** microplate and incubate at room temperature (20 - 25°C) for **60 minutes**. Begin timing after the addition of the last specimen.

8.5 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 **3 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.

8.6 Add **4 drops** (200 µl) of Enzyme Conjugate to each well.

8.7 **Cover** microplate and incubate at room temperature (20 - 25°C) for **30 minutes**.

8.8 Shake out or aspirate and wash each well **5 times** as in step 8.5.

8.9 Add **4 drops** (200 µl) of Colour Substrate to each well.

8.10 **Cover** microplate and incubate at room temperature (20 - 25°C) for **10 minutes**.

8.11 Add **1 drop** (50 µl) 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 Solution.

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

9 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 ≤ 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 ≥ 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.

10 RESULTS

Refer to the enclosed Procedure Card for colour interpretations.

VISUAL

10.1 Read the test results by comparing with the reaction colours on the Procedure Card.
Positive: yellow colour of at least 1+ intensity
Negative: colourless

10.2 Interpretation of visual results:
Positive: If yellow colour of at least 1+ intensity develops in the test well, the sample contains EHSA and the test is positive.
Note: Tests with faint yellow colour (less than 1+) should be repeated.
Negative: A colourless reaction is a negative result and indicates that no EHSA or an undetectable level of EHSA is present in the sample tested.

SPECTROPHOTOMETRIC

10.3 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.

10.5 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.

10.6 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)

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

11 PERFORMANCE LIMITATIONS

The validity of results with the ProSpecT Entamoeba histolytica 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 *Entamoeba histolytica*, 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 Entamoeba histolytica Microplate Assay has been classified as high complexity.

12 EXPECTED VALUES

Entamoeba histolytica infects approximately 12% of the world population[1]. The majority of these infections are asymptomatic, about 10% of infected individuals exhibit clinical symptoms ranging from nonspecific symptoms of gastrointestinal disease to extra-intestinal lesions[1]. In the United States, an average of 3500 cases of amoebiasis per year are reported to the Centres for Disease Control[2]. Recognized high risk groups include travellers, immigrants, migrant workers, immunocompromised persons, persons in mental institutions and sexually active male homosexuals[1]. Among male homosexuals, the nonpathogenic strain of the organism predominates[5]. Asymptomatic carriers of pathogenic strains represent an important reservoir for transmission[1].

13 PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

The performance of ProSpecT Entamoeba histolytica Microplate Assay (EIA) was determined with 268 fresh, frozen faecal specimens. A large clinical reference laboratory provided 105 specimens which were positive for *E. histolytica* by ova and parasite examination (O&P). These specimens had been frozen and stored for ~2-3 years prior to testing in the microplate assay. Of these, 91 were positive by both O&P and EIA and 14 were negative by EIA.

There were 163 fresh, frozen specimens negative by O&P for *E. histolytica*. These specimens were stored for ~6 months. Of these, 48 contained parasites other than *E. histolytica*. All of these 48 specimens were negative for *E. histolytica* by EIA. The remaining 115 specimens contained no parasites by O&P; 114 were negative by EIA and one was positive.

The performance characteristics of ProSpecT Entamoeba histolytica Microplate Assay are summarized below. The true sensitivity of the assay may be higher than given since the occurrence of false positive O&P identifications has been well documented[2].

		O&P +	O&P –	
ProSpecT Entamoeba histolytica	+	91	1	
	–	14	162	
		105	163	268

Sensitivity = 87%
Specificity = 99%
Predictive Value Positive = 99%
Predictive Value Negative = 92%

ANALYTICAL SENSITIVITY

ProSpecT Entamoeba histolytica Microplate Assay detects approximately 40 nanograms/ml of EHSA.

REPRODUCIBILITY

The inter-assay or run-to-run coefficient of variation (CV) of the ProSpecT Entamoeba histolytica Microplate Assay was evaluated by selecting four positive specimens with varying optical density readings. Each sample was tested in 8 wells per day for five consecutive days. The mean inter-assay CV was 4.8%.

Sample	Mean O.D.	Standard Deviation	%CV
1	0.950	0.0210	2.20
2	0.620	0.0143	2.30
3	0.450	0.0355	7.90
4	0.260	0.0179	6.90

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 3.4%.

Sample	Mean O.D.	Standard Deviation	%CV
1	1.086	0.0242	2.23
2	0.685	0.0242	3.54
3	0.481	0.0207	4.31
4	0.289	0.0100	3.50

CROSS-REACTIVITY

ProSpecT Entamoeba histolytica 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 (4)
Blastocystis hominis (5)
Chilomastix mesnili (1)
Cryptosporidium parvum (6)
Dientamoeba fragilis (5)
Endolimax nana (4)
Entamoeba coli (4)
Entamoeba hartmanni (2)
Giardia lamblia (5)
Hookworm (1)
Hymenolepis nana (1)
Iodamoeba butschlii (1)
Isospora spp. (2)
Strongyloides stercoralis (2)
Taenia spp. (1)
Trichuris hominis (2)
Trichuris trichiura (4)

Numbers in parentheses indicate the numbers of specimens tested.

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