Key Code TSMX7834

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ProSpecT Shiga Toxin E.coli (STEC) Microplate Assay

REF R2474048.....48 Tests

REF R2474096.....96 Tests

EN

1. INTENDED USE

The ProSpecT™ Shiga Toxin E. coli (STEC) Microplate Assay is intended for the qualitative detection of Shiga Toxins (Stx1 and Stx2) in aqueous extracts of faecal specimens and broth enriched faecal cultures. The test is intended for use as an aid in the diagnosis of enterohemorrhagic E. coli infections.

2. SUMMARY

Shiga toxin-producing E. coli (STEC) have been recognized as important etiologic agents of diarrhoea and of serious outbreaks and sporadic cases of life-threatening haemorrhagic colitis and haemolytic uremic syndrome [HUS; 2, 3, 13, 14]. Strains of E. coli producing these effects were first described by Konowalchuk et al. who identified a cytotoxin which was cytopathic for Vero cells and was referred to as Verotoxin, VT [6, 7, 8]. The cytotoxin was shown to be closely related to Shiga toxin by O'Brien et al. [9] who referred to the toxin have been identified, Stx1 and Stx2 [9] and Shiga toxin-producing E. coli (STEC) isolates have been shown to produce one or both cytotoxins [15, 16]. E. coli O157:H7 is the most frequently identified EHEC serotype and can be isolated and identified by most clinical laboratories [1, 10, 12, 14]. However, at least 50 serotypes of E. coli have been associated with the production of cytotoxins and development of HUS and/or haemorrhagic colitis [5, 11]. Karmali [4] described a cytotoxin assay for toxin detection but the assay requires considerable time and expertise to confirm the presence of cytotoxin. ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay is an enzyme immunoassay which allows direct detection of Stx1 and Stx2 toxins in stool specimens or in broth enriched faecal cultures.

3. PRINCIPLE OF THE TEST

ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay is a solid phase enzyme immunoassay for the detection of Shiga Toxins. Diluted specimens are added to break-away microplate wells on which rabbit polyclonal anti-Shiga Toxins 1 & 2 are bound. If toxin is present, it is 'captured' by the bound antibody. The wells are incubated and then washed to remove unbound material.

The enzyme conjugate (monoclonal mouse anti-Shiga Toxins 1 & 2 labelled with horseradish peroxidase enzyme) is added. The wells are incubated and then washed to remove unbound enzyme conjugate. In a positive reaction, toxin 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 toxin converts the substrate to a coloured reaction product. Colour development can be detected visually or spectrophotometrically. In a negative reaction, there is no toxin or an insufficient amount of toxin present to bind the enzyme conjugate to the well and no coloured reaction product develops.

4. SYMBOL DEFINITIONS

REF	Catalogue Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitations (Storage temp)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufactured By

5. REAGENTS

The ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay includes sufficient reagents to perform 48 or 96 tests.

REAGENTS	48 / 96 TESTS
Microplate*	**8 wells / strip**
Coated with rabbit polyclonal anti-Shiga Toxins 1 & 2	6 / 12 strips
Enzyme Conjugate *	**1 bottle**
Horseradish peroxidase-labeled mouse monoclonal anti-Shiga Toxins 1 & 2 with 0.01% thimerosal	12 / 25 ml
Positive Control	**1 bottle**
E. coli culture supernatant containing Shiga Toxins 1 & 2 with fetal bovine serum, rabbit serum, and 0.02% thimerosal	4 ml
Negative Control	**1 bottle**
Buffered solution with rabbit serum and 0.02% thimerosal	4 ml
Bacterial Specimen Diluent	**1 bottle**
Buffered solution with rabbit serum and 0.02% thimerosal	110 ml
Wash Buffer	**1 bottle**
10X concentrated buffered solution with 0.1% thimerosal	110 ml
Colour Substrate	**1 bottle**
TMB in buffer	25 ml
Stop Solution	**1 bottle**
1.O N Sulfuric Acid	6 ml

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

6. WARNINGS AND PRECAUTIONS

IVD For *in vitro* Diagnostic Use Only.

6.1. Reagents are provided at the necessary working strength, with the exception of the Wash Buffer concentrate. Do not dilute reagents, except where instructed.

6.2. 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.

6.3. 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.

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

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

6.6. Microplate strips must be stored in the resealable foil pouch to protect microplate wells from moisture.

6.7. 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", 4th Edition.

6.8. Stool samples must be thoroughly mixed prior to specimen processing to ensure accurate representation of the specimen.

DO NOT CONCENTRATE SPECIMENS BEFORE TESTING.

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

6.10. 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.

6.11. Discard used Wash Buffer in appropriate biohazard containers.

6.12. The Wash Buffer (0.1%), Bacterial Specimen Diluent (0.02%), Enzyme Conjugate (0.01%), Positive and Negative Controls (0.02%), contain thimerosal.

6.13. Shiga toxin 1 and the toxin produced by Shigella dysenteriae type I strains (Shiga toxin) are nearly identical. Therefore, ProSpecT Shiga Toxin E. coli (STEC) Mircroplate Assay may be positive whenever a detectable quantity of Shiga toxin is present in the specimen.

7. PREPARATION OF REAGENTS, STORAGE AND HANDLING

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

All reagents, except the Wash Buffer, are supplied in ready-to-use bottles. 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.

Dilute 10x Wash Buffer concentrate to 1x 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.

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 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.

8. COLLECTION OF STOOL SPECIMENS

Fresh specimens (or specimens in Cary-Blair transport media) should be stored at 2-8°C or frozen at -20°C or lower immediately after collection. Specimens for culture should be put into broth within one to two hours after receipt in the laboratory. If cultures cannot be performed within 1-2 hours, fresh or Cary-Blair preserved specimens for culture should be frozen at -20°C or lower. For direct testing of stool specimens, optimal results will be obtained if stools are tested immediately upon receipt in the laboratory. If testing can be conducted within 48 hours of collection for fresh specimens or within 7 days for specimens in Cary-Blair transport media, specimens may be stored at 2-8°C, otherwise store specimens at -20°C or lower. Repeated freeze-thawing may result in degradation of the toxin. Specimens diluted in Bacterial Specimen Diluent may be stored at 2-8°C for up to 48 hours prior to testing.

9. PROCEDURE NOTES

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

10. EQUIPMENT

Required materials provided:

Reagents Transfer pipettes

Microplate Strip Holder and Cover Colour Reaction Procedure Card

Required materials 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 450/630 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

Additional 110mL Wash Buffer REF R2450002

Additional 1L Wash Buffer REF R24500011

11. SPECIMEN PREPARATION FOR ASSAY

A DIRECT STOOL TEST

11.A.1. Add 0.6 ml Bacterial Specimen Diluent to a clean 12 x 75 mm tube.

11.A.2. Mix stool as thoroughly as possible and dilute as follows:

11.A.2.A. Liquid or semi-solid stools: add 0.3 ml (third mark from the tip of the pipette provided) using a transfer pipette. Thoroughly mix stool into the Bacterial Specimen Diluent and leave the transfer pipette in the tube.

11.A.2.B. Solid stools: add 0.3 gm (approximately 6 mm diameter) using an applicator stick. Emulsify stool in the Bacterial Specimen Diluent and place a transfer pipette in the tube.

11.A.2.C. Stools in Cary-Blair transport media should be added directly into the microplate well without further dilution.

11.A.2.D. Vortex stool suspension.

B BROTH METHOD

11.B.1 Inoculate 50 µl or 50 µg (small pea size) fresh stool or stool in Cary-Blair transport media into 5 ml Tryptic Soy Broth (TSB), modified Tryptic Soy Broth (mTSB) or MacConkey Broth.

11.B.2 Incubate at 37°C for 18-24 hours.

11.B.3 Add 0.6 ml Bacterial Specimen Diluent to a clean 12 x 75 mm tube.

11.B.4 Transfer 0.3 ml broth culture into 0.6 ml Bacterial Specimen Diluent using a transfer pipette. Leave the transfer pipette in the tube.

12. TEST METHOD

12.1. 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 the strips. **RETURN UNUSED MICROWELLS TO THE POUCH AND SEAL TIGHTLY TO EXCLUDE MOISTURE.**

12.2. Add 4 drops (200 µl) of Negative Control to the first well. Add 4 drops (200 µl) of Positive Control to a second well.

12.3. Using a transfer pipette, add 4 drops (200 µl) of diluted specimen or undiluted stool in Cary-Blair transport media per well.

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

12.4. Cover the microplate and incubate at room temperature for 60 minutes. Begin timing after the addition of the last specimen.

12.5. 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.

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

12.7. Cover the microplate and incubate at room temperature for 30 minutes.

12.8. Shake out or aspirate and wash each well 5 times as in step 5.

12.9. Add 4 drops (200 µl) Colour Substrate to each well.

12.10. Cover the microplate and incubate at room temperature for 10 minutes.

12.11. Add 1 drop (50 µl) of Stop Solution to each well. Gently tap or vortex the wells until the yellow colour is uniform. Read reactions within 10 minutes after adding Stop Solution.

12.12. 12. Read visually or spectrophotometrically at 450 nm or 450/630-650 nm.

13. 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 cutoff.

The optical density (O.D.) of the Negative Control should be < 0.100 for single wavelength and < 0.070 for dual wavelength and should be colorless 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 >0.500 for both single and dual wavelengths, 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.

14. RESULTS

Refer to the enclosed Procedure Card for colour interpretations.

VISUAL:

14.1. Read the test results by comparing with the reaction colors on the procedure card.

Positive: yellow colour of at least 1+ intensity

Negative: colorless

Indeterminant: faint yellow colour, less than the 1+ reaction

14.2. Interpretation of visual results:

Positive: If yellow colour of at least 1+ intensity develops in the test well, the sample contains either Stx1 or Stx2 or both and the test is positive.

Negative: A colorless reaction is a negative result and indicates that no Stx1 or Stx2 or an undetectable level of toxins is present in the sample tested.

Indeterminant: If faint yellow colour that is less than the 1+ reaction develops, the test is indeterminant.

Indeterminant results should be repeated.
If the repeat test results are positive, the specimen is positive.
If the repeat test results are negative, the specimen is negative.
If the repeat test results remain indeterminant another specimen should be obtained and tested.

SPECTROPHOTOMETRIC:

14.3. Read results at either single (450 nm) or dual (450/630 - 650 nm) wavelength.

14.4. Read the test results:

14.4.A. Single Wavelength

Positive: O.D. > 0.150
Negative: O.D. <0.100
Indeterminant: O.D. 0.100-0.150.

14.4.B. Dual Wavelength

Positive: O.D. >0.100
Negative: O.D. <0.070
Indeterminant: O.D. 0.070-0.100

14.5. Interpretation of spectrophotometric results:

Positive: An O.D. reading greater than 0.150 (single wavelength) or greater than 0.100 (dual wavelength) is positive and indicates the presence of Stx1 and / or Stx2.

Negative: An O.D. reading less than 0.100 (single wavelength) or less than 0.070 (dual wavelength) is a negative result and indicates that no Stx1 or Stx2 or both or an undetectable level of the toxin is present in the sample tested.

Indeterminant: O.D. readings of 0.100-0.150 (single wavelength) or 0.070-0.100 (dual wavelength) are indeterminant.

Indeterminant results should be repeated.
If the repeat test results are positive, the specimen is positive.
If the repeat test results are negative, the specimen is negative.
If the repeat test results remain indeterminant another specimen should be obtained and tested.

Note: Any wells that are clear visually but give an O.D. reading that is inconsistent with 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 these, wipe the underside of the wells and read the O.D. again. If the discrepancy between visual and OD readings persist, repeat the test.

15. LIMITATIONS OF THE PROCEDURE

15.1. The validity of results with the ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay depends on the control reaction performing as expected. See Quality Control section.

15.2. A negative test result does not exclude the possibility of the presence of Shiga Toxins, 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.

15.3. As with all IN VITRO diagnostic tests, results should be interpreted by the clinician in conjunction with clinical findings and/or other laboratory results.

15.4. 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 Stool Specimens section.

ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay has been classified as high complexity.

16. EXPECTED VALUES

At least 50 different serotypes of Shiga toxin-producing E. coli (STEC) have been associated with the production of cytotoxins and development of life-threatening haemorrhagic colitis and haemolytic uremic syndrome (HUS) - a major complication resulting in renal failure, thrombocytopenia and anaemia. Although young children are at high risk of developing HUS as a result of contracting a Shiga toxin-producing E. coli infection other selected subpopulations may also be at risk from an STEC infection such as the elderly and immunocompromised patients.

As seen in the results of the clinical trials with the ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay a variety of different serotypes were present in the population tested. Testing for STEC infections should not be limited to tests that are specific for O157:H7 only. It should also be noted that there is not always a direct correlation between the results of direct and broth enriched testing. This may be because of, but not limited to, the following:

16.4.A. Toxin can be present in the stool at a detectable level but STEC cells are no longer viable for culturing;

16.4.B. Isolated E. coli O157 strains from stool culture of patients is inversely related to the interval between the onset of diarrhoea and the microbiological culture [17];

16.4.C. Toxin may be present in the stool at a level undetectable by the assay but STEC cells are still viable for culturing;

16.4.D. Antimicrobial therapy may impact the culturability of STEC cells.

17. PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity:

The ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay was evaluated at three geographically distinct clinical sites in the United States, Canada and Germany. The sites were a Metropolitan Hospital in Virginia, USA, a Children's Hospital in Toronto, Canada and a large Microbiology Institution in Germany. Patient populations represented in the specimen pool were symptomatic patients in normal adult and paediatric prevalence populations.

Specimens were submitted unpreserved and were tested in the ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay as fresh specimens and as overnight enrichment cultures. All specimens were also tested by a cytotoxin assay (CTA) for the presence of Shiga toxins. Shiga toxin positive specimens were isolated and serotyped (see Table 3). An Investigational Use Only Polymerase Chain Reaction (PCR) amplification assay for the presence of the Shiga toxin gene was performed on all positive and discrepant results.

The results at each of the test sites are presented in Tables 1 and 2. In the Direct Testing method there were 20 positive specimens (EIA+, CTA+, PCR+). There were 3 EIA-, CTA+, PCR+ specimens. There were 5 EIA+, CTA-, PCR- specimens. All indeterminant results were resolved as true negatives. 354 specimens were negative by both methods (EIA-, CTA-).

The overall initial and resolved performance of the ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay are presented below in Table 1.

Table 1: ProSpecT Shiga Toxin E.*coli* (STEC) Microplate Assay compared with a Cytoxin Assay on Direct Stool specimens

Direct Specimen Testing

	Initial Results				Resolved Results*		
ProSpecT	Cytoxin Assay			ProSpecT	Cytoxin Assay		
Trial Site 1	+	-	Ind.		+	-	Ind.
+	12	2	0	+	12	2	0
-	1	156	4	-	1	161	0
Ind.	0	0	1	Ind.	0	0	0
Total Specimens:	176				176		
Sensitivity:	92.3%				92.3%		
Specificity:	98.7%				98.7%		

	Initial Results				Resolved Results*		
ProSpecT	Cytoxin Assay			ProSpecT	Cytoxin Assay		
Trial Site 2	+	-	Ind.		+	-	Ind.
+	8	3	0	+	8	3	0
-	2	186	6	-	2	193	0
Ind.	0	0	1	Ind.	0	0	0
Total Specimens:	206				206		
Sensitivity:	80.0%				80.30%		
Specificity:	98.4%				98.5%		

	Initial Results				Resolved Results*		
ProSpecT	Cytoxin Assay			ProSpecT	Cytoxin Assay		
Combined	+	-	Ind.		+	-	Ind.
+	20	5	0	+	20	5	0
-	3	342	10	-	3	354	0
Ind.	0	1	1	Ind.	0	0	0
Total Specimens:	382				382		
Sensitivity:	87.0% (66.4-97.2)				87.0% (66.4-97.2)		
Specificity:	98.6% (96.7-99.5)				98.6% (96.6-99.5)		
Correlation:	95.0% (92.3-97.0)				97.9% (95.9-99.1)		

Numbers in parenthesis are 95% Confidence intervals.

*The resolved tables show the results of repeat EIA testing on the specimens that were initially indeterminant.

As shown in Table 1 there is a 97.9% correlation in the direct specimen testing method for the resolved data between the ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay and cytotoxin assay when the results of both sites are combined. In the Broth Enriched Culture method there were 63 positive specimens (EIA+, CTA+, PCR+). There were 3 EIA-, CTA+, PCR- specimens. Upon repeat CTA testing 2 of 3 were resolved as true negatives. There were 10 EIA+, CTA-, PCR- specimens. All indeterminant results were resolved as true negatives after repeat testing. 771 specimens were negative by both methods (EIA-, CTA-). The overall initial and resolved performance of the ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay is presented in Table 2.

Table 2. ProSpecT Shiga Toxin E. *coli* (STEC) Microplate Assay Compared with a Cytotoxin Assay on Broth Enriched Culture Specimens.

Broth Enrichment Cultures

	Initial Results				Resolved Results*		
ProSpecT	Cytoxin Assay			ProSpecT	Cytoxin Assay		
Trial Site 1	+	-	Ind.		+	-	Ind.
+	12	1	0	+	12	1	0
-	0	160	3	-	0	163	0
Ind.	0	0	0	Ind.	0	0	0
Total Specimens:	176				176		
Sensitivity:	100%				100%		
Specificity:	99.4%				99.4%		

	Initial Results				Resolved Results*		
ProSpecT	Cytoxin Assay			ProSpecT	Cytoxin Assay		
Trial Site 2	+	-	Ind.		+	-	Ind.
+	9	0	0	+	9	0	0
-	1	192	4	-	1	196	0
Ind.	0	0	0	Ind.	0	0	0
Total Specimens:	206				206		
Sensitivity:	90.0%				90.0%		
Specificity:	100%				100%		

	Initial Results				Resolved Results*		
ProSpecT	Cytoxin Assay			ProSpecT	Cytoxin Assay		
Trial Site 3	+	-	Ind.		+	-	Ind.
+	42	9	0	+	42	9	0
-	2**	410	0	-	0	412	0
Ind.	0	0	0	Ind.	0	0	0
Total Specimens:	463				463		
Sensitivity:	100%				100%		
Specificity:	97.9%				97.9%		

	Initial Results				Resolved Results*		
ProSpecT	Cytoxin Assay			ProSpecT	Cytoxin Assay		
Combined	+	-	Ind.		+	-	Ind.
+	63	10	0	+	63	10	0
-	3	762	7	-	1	771	0
Ind.	0	0	0	Ind.	0	0	0
Total Specimens:	845				845		
Sensitivity:	95.5% (87.3-99.1)				98.4% (91.6-100)		
Specificity:	98.7% (97.6-99.4)				98.7% (97.7-99.4)		
Correlation:	97.6% (96.4-98.5)				98.7% (97.7-99.3)		

Numbers in parentheses are 95% confidence intervals.

*The resolved tables show the results of repeat EIA testing on the specimens that were initially indeterminant.

** Note that in Trial Site 3 there were 2 initially CTA false positive results that were negative upon repeat CTA Testing.

As shown in Table 2 there was 98.7% correlation in the broth enriched culture method for the resolved data between the ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay and cytotoxin assay when the results of all sites were combined.

Various serotypes of Shiga toxin-producing E. coli strains have been tested in the ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay and found to be reactive either from direct stool testing or from enriched broth. Below is a list of the serotypes tested, the number of serotype-specific specimens and the type of toxin produced by each strain, where known.

Table 3. Number of Different Serotypes of Shiga Toxin producing E. coli strains isolated from positive specimens.

Serotype	Number of strains isolated					
Serotypes	Stx1	Stx2	Stx1&2	Stx2c	Unknown	Total
O8:H9		1				1
O26:H11	7	2	1		3	13
O30		1				1
O88:H5		1				1
O91	2	1				3
O103:H2	1					1
O111:NM	2	1				3
O118	2					2
O128	2					2
O145	1	1				2
O153:H2	1	1				2
O157:H7		7	9	2		18
O166	1					1
Total Toxin Types	19	16	10	2	3	50

Table 4. Shiga Toxin Positive Tests by Stool Consistency

	Stool Consistency					
	Watery	Soft	Mucus	Watery/ Mucus	Soft/ Mucus	Bloody
No. of stools	119	134	41	17	50	30
Shiga Toxin +	8	5	5	0	2	7
Cytotoxin +	10	5	5	1	3	7

Anaytical Sensitivity: The ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay detects approximately 52 pg/ml of Stx1 and 126 pg/ml of Stx2.

Reproducibility: The inter-assay coefficient of variation (CV) of the ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay was evaluated by selecting one negative and three positive specimens with varying optical density readings. Each specimen was tested in 24 wells per run in three consecutive runs. The mean inter-assay CV was 8.69%

Sample	Mean O.D.	Standard Deviation	%CV
1	0.065	0.0101	15.61
2	0.264	0.0176	6.67
3	0.675	0.0270	4.00
4	0.830	0.0703	8.48

The intra-assay CV was evaluated by testing 24 wells with each of 4 specimens. The Mean intra-assay CV was 4.59%

Sample	Mean O.D.	Standard Deviation	%CV
1	0.069	0.0055	8.01
2	0.274	0.0134	4.90
3	0.563	0.1490	2.65
4	1.226	0.0340	2.78

Cross Reactivity: There was no cross reaction when a variety of organisms of the human colonic microflora were tested in the ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay. Tests were conducted by seeding the organisms listed below into Shiga toxin negative and positive stools. Bacteria were seeded at concentrations >1 x 107 CFU/ml of stool. Non Shiga toxin-producing E. coli pathogenic strains (EPEC, ETEC, EIEC) were also tested in the ProSpecT assay and were negative.

Campylobacter jejuni ATCC® 29428
Klebsielia pneumoniae ATCC® 27736
Citrobacter braaki ATCC® 43162
Pseudomonas aeruginosa ATCC® 27853
Enterobacter cloacae ATCC® 13047
Proteus vulgarus ATCC® 33420
Enterococcus faecalis ATCC® 49149
Salmonella typhimurium SA972229
Escherichia coli, VT negative, ATCC® 25922
Serratia liquefacians ATCC® 27592
Escherichia coli, EPEC, ATCC® 12014 (O55:NM)
Shigella dysenteriae ATCC® 49347
Escherichia coli, EPEC, ATCC® 33780 (O111:NM)
Shigella flexneri ATCC® 25929
Escherichia coli, ETEC/ EPEC, ATCC® 43887 (O111:NM)
Shigella sonnei ATCC® 25931
Escherichia coli, EIEC, ATCC® 43893 (O124:NM)
Staphylococcus aureus ATCC® 25923
Escherichia coli ATCC® 33660
Yersinia enterocolitica ATCC® 2371516

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IFU X7834, Revised May 2012
Printed in the UK

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