

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

Direct Specimen Testing							
Initial Results			Resolved Results*				
ProSpecT	Cytotoxin Assay		ProSpecT	Cytotoxin 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%			

Direct Specimen Testing							
Initial Results			Resolved Results*				
ProSpecT	Cytotoxin Assay		ProSpecT	Cytotoxin 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%			

Direct Specimen Testing							
Initial Results			Resolved Results*				
ProSpecT	Cytotoxin Assay		ProSpecT	Cytotoxin 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 indeterminate.

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 indeterminate 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	Cytotoxin Assay		ProSpecT	Cytotoxin 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%			

Broth Enrichment Cultures							
Initial Results			Resolved Results*				
ProSpecT	Cytotoxin Assay		ProSpecT	Cytotoxin 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%			

Broth Enrichment Cultures							
Initial Results			Resolved Results*				
ProSpecT	Cytotoxin Assay		ProSpecT	Cytotoxin 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%			

Broth Enrichment Cultures							
Initial Results			Resolved Results*				
ProSpecT	Cytotoxin Assay		ProSpecT	Cytotoxin 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 indeterminate.

** 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					Total
	Stx1	Stx2	Stx1&2	Stx2c	Unknown	
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

Analytical 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

Klebsiella pneumoniae ATCC® 27736

Citrobacter braaki ATCC® 43162

Pseudomonas aeruginosa ATCC® 27853

Enterobacter cloacae ATCC® 13047

Proteus vulgaris ATCC® 33420

Enterococcus faecalis ATCC® 49149

Salmonella typhimurium SA972229

Escherichia coli, VT negative, ATCC® 25922

Serratia liquefaciens 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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