

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE**

A. 510(k) Number: K031367

B. Analyte: *Escherichia coli*

C. Type of Test: Qualitative, immunochromatographic rapid test

D. Applicant: EMD Chemicals, Inc.

E. Proprietary and Established Names: Duopath® Verotoxins GLISA Test

F. Regulatory Information:

1. Regulation section: *Escherichia coli* serological regents, 21 CFR 866.3255
2. Classification: Class I
3. Product code: GNA
4. Panel: Microbiology (83)

G. Intended Use and Indication for Use:

1.Intended use(s): In vitro diagnostic test for qualitative detection of verotoxins (Shiga-like toxins[SLT] I and II from *E.coli*

1.Indication(s) for use: The Duopath® Verotoxins GLISA test is a rapid test for the qualitative identification of Verotoxins I and II (Shiga-like toxins) produced by *E. coli* isolated in cultures derived from clinical specimens. The identification aids in the diagnosis of diseases caused by enterohemorrhagic *E. coli* infections.

H. Device Description: Duopath Verotoxin is an immunochromatographic rapid test based on the GLISA method (Gold-labeled Immunosorbent Assay). Duopath Verotoxin enables the detection of verotoxin (synonymous: Shiga-like toxins, SLT's) variants I and II from bacterial culture isolates. The test device has a circular sample port, and an oval shaped test (VT1, VT2) and control © window. The device includes a highly specific gold-marked monoclonal antibodies, which can bind verotoxin I and II upon application of verotoxin-containing sample material to the sample port.

I. Substantial Equivalence Information:

1. Predicate device name(s): Meridian Premier EHEC
2. Predicate K number(s): K950167 and K953362

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
1. Intended Use	Detection of verotoxins (Shiga-like toxins[SLT] I and II from <i>E.coli</i>)	Detection of verotoxins (Shiga-like toxins[SLT] I and II from <i>E.coli</i>)
2. Configuration	ELISA (enzyme-linked Immunosorbent assay) in which the antigen is embedded between a capture antibody and a detection antibody and incorporates monoclonal antibodies. Specific for Verotoxin I and II.	ELISA (enzyme-linked Immunosorbent assay) in which the antigen is embedded between a capture antibody and a detection antibody and incorporates monoclonal antibodies. Specific for Verotoxin I and II.
3. Sample	Stool	Stool
Differences		
Item	Device	Predicate
1. Type	Lateral flow immunochromatographic rapid test based on gold-labeled antibodies.	ELISA
2. Antibody	Immobilized and dried on the surface of gold particles.	Immobilized and dried in a polystyrene surface.
3. Color Reaction	Mediated by the red color of the gold particles	Mediated by the peroxidase enzyme
4. Test Time	20 minutes	3 to 5 hours

J. Standard/Guidance Document Referenced : Not Applicable

K. Test Principle: The Duopath® Verotoxin uses GLISA (gold labeled immunosorbent assay) methodology. GLISA uses monoclonal antibodies immobilized on gold particles. The gold-labeled monoclonal antibodies flow in complexes with the verotoxin over a cellulose nitrate membrane and attach to immobilized monoclonal antibodies, which are also highly specific for the verotoxin I and verotoxin II on the membrane. The sample reacts with gold coated particles that have been applied to the label pad. This mixture migrates chromatographically along the length of the test strip and interacts with the immobilized monoclonal antibodies. If the sample contains verotoxin, a colored line will appear in the test line region and control position indicating a positive result. If the sample does not contain verotoxin, a red colored line will only appear in the control region indicating a negative result. To serve as a procedural control, a red colored line will always appear at the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

L. Performance Characteristics :

1. Analytical performance:

- a. *Precision/Reproducibility:* Reproducibility studies evaluated inter-lot, inter/intra- site and inter-laboratory variability. Assay performance was evaluated by testing 9 specimens three times over three days at three sites. Samples consisted of three negative, three low positives and three strong positives. The result of combined with-in run and total imprecision are shown within the package insert. Results were presented according to the NCCLS standards.
- b. *Linearity/assay reportable range:* Not Applicable
- c. *Traceability, Calibrators, and Controls:* Not Applicable
- d. *Analytical sensitivity:* The lower limits of detection were calculated as 25 ng/mL for VT1 and 62.5 ng/mL for VT2. Solutions containing verotoxins 1 and 2 were mixed 1 + 1 and applied onto the Duopath assay. Parallel testing was performed on 4 production batches and the results were reported as dose response.
- e. *Analytical specificity:* There was no cross-reactivity when a variety of organisms, which included *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Salmonella*, and *Shigella*, were tested with the Duopath® Verotoxin assay. The methodology used for the cross-reactivity study did not allow a determination for the numerical concentration of the potentially cross-reactive organisms. Organisms were inoculated onto Sorbitol-Mackey (SMAC) agar. The inoculum was prepared using a Dacron swab which was swept across the areas of growth on the plates. The swabs were incubated in appropriate media (18/24 hr for EHEC) or in distilled water treated with polymyxin B (30 minutes for Duopath®). The number of isolates tested and results are listed below.

Organism	No. isolates tested	EHEC	Duopath
<i>Ps. Aeruginosa</i>	10	Negative	Negative
<i>K. pneumoniae</i>	10	Negative	Negative
<i>Enterobacter</i> sp.	10	Negative	Negative
<i>Proteus</i> sp.	10	Negative	Negative
<i>Aeromonas</i> sp.	3	Negative	Negative
<i>Serratia</i> sp.	5	Negative	Negative
<i>Shigella</i> sp.	3	Negative	Negative
<i>Salmonella</i>	7	Negative	Negative

- f. *Analytical characterization of cut-off:* Assay cut-off was determined by reading against a color intensity chart. The intensity of the color on the device was compared to the chart and scored using a scale from 0 – 10.

2. Comparison studies using clinical specimens:

Method comparison: Two hundred ninety retrospective and prospective stool specimens were evaluated with the Duopath® Verotoxin and the Premier™ EHEC assays. Of the 290, 41 were retrospective and 249 were prospective specimens. Results were reported as 100% positive agreement for the retrospective study, and 100% positive agreement and 99.6 % negative agreement

with the prospective study. The firm reported no false positive result and one false negative result.

DUOPATH® REFERENCE METHOD*	VEROTOXIN Fresh Specimen		Totals
	Positive	Negative	
Positive	5	0	5
Negative	1	243	244
Totals	6	243	249
% agreement +	100%	5/5	
% agreement –	99.6%	243/244	

DUOPATH® REFERENCE METHOD*	VEROTOXIN Frozen Specimen		Totals
	Positive	Negative	
Positive	41	0	41
Negative	0	0	0
Totals	41	0	41
% agreement +	100%	41/41	
% agreement –	No Negative Results		

- a. *Matrix description and comparison:* Not Applicable

M. Conclusion: Performance demonstrated that the Duopath® Verotoxin GLISA assay to be substantially equivalent to other legally marketed devices performing the same testing.