

VIDAS® *E. coli* O157 (ECO) and O157:H7 Plate Method
Pre-collaborative Study Report: AOAC Performance Tested MethodSM
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Abstract

The VIDAS *E. coli* O157 (ECO) assay was evaluated according to AOAC Guidelines and Performance Tested Method program requirements. There are two VIDAS ECO methods: an 8-hour method using modified Trypticase Soy Broth (mTSB) without novobiocin and a 24-hour method using mTSB with 2% novobiocin for 8 hours followed by MacConkey broth with cefixime and sodium tellurite (CT-MAC) for 18-24 hours. Both methods were shown to be equivalent to the USDA FSIS method for the analysis of ground beef in two precollaborative studies. O157:H7 Chromogenic ID medium and CT-SMAC medium were essentially equivalent in the isolation of O157:H7 colonies. However, O157:H7 colonies were easier to select from the chromogenic plate.

Materials and Methods

Test Organism: One *E. coli* O157:H7 isolate (SLR 1883) was used in the study. Culture for inoculation was grown in TSB at 35°C for 18-24 hours. Numbers of colony forming units were determined on TSA incubated at 35°C for 24 hours. Broth culture was stored at 4°C prior to product inoculation. Dilutions were prepared with Butterfield's phosphate buffer (0.3 mM, pH 7.2).

Test Product and Inoculation: Two production lots of ground beef were purchased from a local grocer for inoculation and testing. One was used for the 8 hr evaluation and the other for the 24 hr evaluation. One to 2 mL of an appropriate dilution of the 24-hour broth culture was added to 2,500 grams of beef and mixed thoroughly to obtain the desired levels of inoculation for each product lot. After inoculation, samples were stored for 48 h at 4°C to adapt the inoculum to the product.

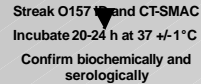
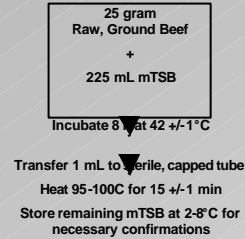
Methods: Twenty 50 gram samples of inoculated ground beef and five 50 gram samples of uninoculated ground beef were analyzed for *E. coli* O157:H7 by the USDA cultural method and the VIDAS ECO 8 h method. Twenty 50 gram samples of inoculated ground beef and five 50 gram samples of uninoculated ground beef were analyzed by the USDA cultural method and the VIDAS ECO 24 h method. From each 50 gram test sample, paired 25 gram test portions were prepared – one for the USDA method and one for the respective VIDAS method.

For comparative analysis, all samples were streaked to O157:H7 ID, chromogenic medium (bioMérieux ref 42605) with CT (Cefixime-Telluritemixture bioMérieux ref. 42606), and CT-SMAC (sorbitol MacConkey with 0.05 mg/L Cefixime and 2.5 mg/L Tellurite) medium. Typical colonies were confirmed using biochemical and serological determinations according to the FSIS method.

Note: VIDAS ECO package insert instructions recommend O157:H7 confirmation from either O157:H7 ID or CT-SMAC.

Two method comparison trials were conducted (Study 1 and Study 2).

8 H ECO Method



24 H ECO Method

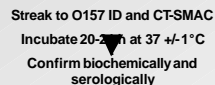
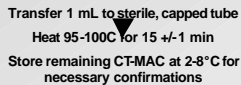
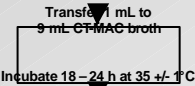
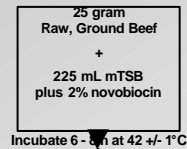


Table 1. Comparison of 8 hr ECO (O157ID) and USDA culture methods for detection of *E. coli* O157:H7 in ground beef.

<i>E. coli</i> O157:H7 MPN/g	8 hr ECO with O157ID				Culture method	X ²	specificity		Agreement %
	Total samples	total + samples	presumptive	confirmed			kit	false pos kit	
STUDY 1									
<0.003	5	0	0	0	0	-	100	0	100
0.043	20	17	15	15	8	3.27	100	0	45
STUDY 2									
<0.003	5	0	0	0	0	-	100	0	100
0.043	20	13	10	10	6	0.90	100	0	50

Table 2. Comparison of 8 hr ECO (CT SMAC) and USDA culture methods for detection of *E. coli* O157:H7 in ground beef.

<i>E. coli</i> O157:H7 MPN/g	8 hr ECO with CT SMAC				Culture method	X2	specificity		Agreement %
	Total samples	total + samples	presumptive	confirmed			kit	false pos kit	
STUDY 1									
<0.003	5	0	0	0	0	-	100	0	100
0.043	20	17	15	15	8	3.27	100	0	45
STUDY 2									
<0.003	5	0	0	0	0	-	100	0	100
0.043	20	13	10	10	6	0.90	100	0	50

Table 3. Comparison of 24 hr ECO (O157 ID) and USDA culture methods for detection of *E. coli* O157:H7 in ground beef.

<i>E. coli</i> O157:H7 MPN/g	24 hr ECO with O157ID				Culture method	X2	specificity		Agreement %
	Total samples	total + samples	presumptive	confirmed			kit	false pos kit	
STUDY 1									
<0.003	5	0	0	0	0	-	100	0	100
0.015	20	13	8	8	7	0.00	100	0	45
STUDY 2									
<0.003	5	0	0	0	0	-	100	0	100
0.043	20	15	12	12	6	2.08	100	0	40

Table 4. Comparison of 24 hr ECO (CT SMAC) and USDA culture methods for detection of *E. coli* O157:H7 in ground beef.

<i>E. coli</i> O157:H7 MPN/g	24 hr ECO with CT SMAC				Culture method	X2	specificity		Agreement %
	Total samples	total + samples	presumptive	confirmed			kit	false pos kit	
STUDY 1									
<0.003	5	0	0	0	0	-	100	0	100
0.015	20	13	8	8	7	0.00	100	0	45
STUDY 2									
<0.003	5	0	0	0	0	-	100	0	100
0.043	20	15	12	12	6	2.08	100	0	40

Data Analysis: The number of samples found positive by VIDAS 8 h or VIDAS 24 h and negative by USDA procedure vs. number of samples positive by USDA and negative by VIDAS 8 h or VIDAS 24 h were compared using McNemar's method for paired analysis. A X² value of

3.84 was indicative of a significant difference at the 5% probability level.

Results and Discussion

Method Comparison -- STUDY 1:

Results for samples analyzed by VIDAS 8 h methods and USDA method are presented in Tables 1 and 2. At an MPN value of 0.043/g (1.08/25g), there were 8 positive samples by the USDA method and 15 presumptive positives by the VIDAS 8-hour method (45% method agreement for inoculated samples). Sixteen of the VIDAS sample enrichment broths were confirmed positive from both CT-O157:H7 ID agar and CT-SMAC agar. Fifteen of the sixteen confirmed positives were positive by the VIDAS assay, resulting in one false negative for the VIDAS method and no false positives.

Results for samples analyzed by the VIDAS 24 h methods and USDA method are presented in Tables 3 and 4. At an MPN value of 0.015/g (0.38/25g), there was 45% agreement between the two assays for the inoculated samples. There was one more positive sample by the VIDAS assay than the USDA assay. All 8 of the VIDAS sample enrichment broths were confirmed positive from both CT-O157:H7 and CT-SMAC agar.

Method Comparison -- STUDY 2:

Results from ground beef testing in Study 2 are presented in Tables 1 - 4. At an MPN value of 0.043/g (1.08/25 g), there were 6 positives by the USDA method, 10 positives by the VIDAS 8-hour method and 12 positives by the VIDAS 24-hour method. The two isolation agars used in the confirmation procedure for VIDAS sample enrichment broths yielded equivalent results. All uninoculated control samples were negative by both assays. The method agreement in the inoculated samples was 50% and 40% for the 8-hour and 24-hour methods, respectively, but the methods were not significantly different (p<0.05) by Chi square analysis.

Summary

The number of positive VIDAS samples was compared to USDA's cultural method. Although the total number of positive samples was higher with the VIDAS methods than USDA method, the methods were not significantly different (p<0.05) by Chi square analysis. All uninoculated control samples were negative by both assays. Recovery of presumptive positive isolates for O157:H7 on CT-O157:H7 ID Chromogenic media and CT-SMAC were essentially equivalent. However, the bluish-green O157:H7 colonies were easier to select from the chromogenic plate.

Recommendation

It is recommended that further evaluations be performed to evaluate the VIDAS ECO method for AOAC RI approval.