

**Interpretation Number: 09-XV-.03-100**

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**National Shellfish Sanitation Program  
U.S. Food and Drug Administration  
Shellfish Safety Team  
Division of Cooperative Programs  
Office of Compliance  
Date: February 14, 2001  
Revised: December 8, 2002**

**Model Ordinance** Chapter XV. 03. L. (1) (c)

**Reference:** NSSP Guidance Documents

Approved NSSP Laboratory Tests

**Key Words:**

Sample Volume, Fecal Coliform Counts, MPN Table and Count Range

**Question:**

What sample volume is inoculated in the 12-tube, single dilution MPN test for end product deperated shellfish samples?

How are fecal coliform counts determined using the 12-tube, single dilution MPN test for end product deperated shellfish?

**Interpretation:**

Two (2) mls (1 gram) of a 1:1 dilution of shellfish homogenate is inoculated into each tube of single strength lauryl tryptose presumptive broth in the 12-tube, single dilution MPN test for end product deperated shellfish samples. Inoculated tubes are incubated in an air incubator at 35°C for 24 hours. Any gas positive presumptive broth tubes are then subcultured to EC medium and incubated in a water bath at 44.5°C for 24 hours. The presence of any amount of gas or effervescence in the EC tubes constitutes a positive test. Fecal coliform counts are read from the MPN Table below and reported as MPN/100 grams.

MPN Table for End-product Depurated Shellfish Samples	
Number of Positive Tubes	MPN/100 grams
0	< 9.0
1	9.0
2	18
3	29
4	41
5	54
6	70
7	88
8	110
9	139
10	179
11	248
12	>248
Count range 9 to 248	

**Rationale:**

The use of the 12-tube, single dilution MPN test for end-product depurated shellfish was established as an acceptable method of analysis with the ISSC's adoption of the rewrite of Model Ordinance, Chapter XV, the Depuration Chapter in 1998. However, no specific guidance was provided on sample volumes to be examined or how fecal coliform counts were to be determined. Since the volume of sample inoculated in a single dilution MPN test controls the range of counts that can be determined, it is essential that an appropriate volume be inoculated to encompass the count range prescribed as the critical limits for depuration plant performance listed for all shellfish species encountered.

The inoculation of two (2) ml (yielding 1 gram) of sample from an initial 1:1 dilution of shellfish homogenate into each tube of the 12-tube, single dilution MPN produces a range of counts from 9 to 248. This range is sufficient to cover the critical limits of performance of all shellfish types listed in Chapter XV. 03. L (1) (c).

**Remarks:**

Comments received from the review of the draft version of Interpretation 03-XV-.03-100 indicated that the content of the Interpretation was too broad to be dealt with effectively in a single Interpretation. For this reason, this second Interpretation was developed from information presented in the first concerning the correct application of the single dilution MPN test to end product depurated shellfish.

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