

**AOAC TDLM Method Verification Workshop**  
**Detection of *Salmonella* in Cloves**



**122<sup>nd</sup> AOAC**  
**ANNUAL MEETING & EXPOSITION**

## **Detection of *Salmonella* in Cloves (AOAC 967.26)**

### **Activity:**

The lab is verifying the method for the detection of *Salmonella* in cloves by cultural method. Use the ALACC Method Verification Guide and information from the AOAC collaborative study to plan how the lab should verify the method.

### ***Verification Purpose***

The lab has to provide evidence that it can achieve the performance characteristics stated in the standardized method (AOAC 967.26).

### ***AOAC Collaborative Study Information***

Results are presented from a collaborative study of a method for the identification of *Salmonella* in whole cloves and clove powder. Two parameters were evaluated. The first parameter examines the effect of matrix. Whole cloves and clove powder were inoculated with 100 CFU/g of *Salmonella*, and samples were tested at 10-fold dilutions of product in three different enrichment media by 6 laboratories participating in the trial (tables 1-3). The second parameter evaluates the limit of detection by inoculating three different concentrations of *Salmonella* (10, 100 and 1,000 CFU/g) into samples of whole cloves (tables 4-6). The cloves were diluted 1:1,000 to overcome the matrix effects. Samples were analyzed by 6 labs in triplicate. The LOD was then calculated using the Spearman Karber calculation presented in the spreadsheet by Dr. Anthony Hitchins.

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Table 1. Interlaboratory study results for *Salmonella* in whole cloves and clove powder enriched in Trypticase Soy Broth (TSB)

Food	Product Dilution	Lab 1 result	Lab 2 result	Lab 3 result	Lab 4 result	Lab 5 result	Lab 6 result
Whole cloves	1:10	Negative	Negative	Negative	Negative	Negative	Negative
	1:100	Positive	Negative	Negative	Positive	Negative	Negative
	1:1,000	Positive	Positive	Positive	Positive	Positive	Positive
	1:10,000	Negative	Negative	Negative	Positive	Negative	Positive
Clove powder	1:10	Negative	Negative	Negative	Negative	Negative	Negative
	1:100	Negative	Negative	Negative	Negative	Negative	Negative
	1:1,000	Negative	Positive	Positive	Positive	Negative	Positive
	1:10,000	Positive	Positive	Negative	Positive	Positive	Positive

Table 2. Interlaboratory study results for *Salmonella* in whole cloves and clove powder enriched in Trypticase Soy Broth (TSB) with added K<sub>2</sub>SO<sub>3</sub>

Food	Product Dilution	Lab 1 result	Lab 2 result	Lab 3 result	Lab 4 result	Lab 5 result	Lab 6 result
Whole cloves	1:10	Negative	Negative	Negative	Negative	Negative	Negative
	1:100	Negative	Negative	Negative	Positive	Negative	Negative
	1:1,000	Positive	Negative	Positive	Positive	Negative	Positive
	1:10,000	Negative	Negative	Negative	Negative	Negative	Negative
Clove powder	1:10	Negative	Negative	Negative	Negative	Negative	Negative
	1:100	Negative	Negative	Negative	Negative	Negative	Negative
	1:1,000	Negative	Positive	Positive	Positive	Negative	Positive
	1:10,000	Negative	Negative	Negative	Negative	Negative	Negative

Table 3. Interlaboratory study results for *Salmonella* in whole cloves and clove powder enriched in Lactose Broth

Food	Product Dilution	Lab 1 result	Lab 2 result	Lab 3 result	Lab 4 result	Lab 5 result	Lab 6 result
Whole cloves	1:10	Negative	Negative	Negative	Negative	Negative	Negative
	1:100	Negative	Negative	Negative	Negative	Negative	Negative
	1:1,000	Negative	Negative	Negative	Positive	Negative	Positive
	1:10,000	Negative	Negative	Negative	Negative	Negative	Negative
Clove powder	1:10	Negative	Negative	Negative	Negative	Negative	Negative
	1:100	Negative	Negative	Negative	Negative	Negative	Negative
	1:1,000	Negative	Negative	Negative	Negative	Negative	Negative
	1:10,000	Negative	Negative	Negative	Negative	Negative	Negative

## AOAC TDLM Method Verification Workshop Detection of *Salmonella* in Cloves

Table 4: 10 CFU/25 g inoculum

Food	Sample Replicate	Lab 1 result	Lab 2 result	Lab 3 result	Lab 4 result	Lab 5 result	Lab 6 result
Whole cloves	1	Negative	Negative	Negative	Negative	Negative	Negative
1:1,000	2	Negative	Negative	Negative	Negative	Negative	Negative
	3	Negative	Negative	Negative	Negative	Negative	Negative

Table 5: 100 CFU/25 g inoculum

Food	Sample Replicate	Lab 1 result	Lab 2 result	Lab 3 result	Lab 4 result	Lab 5 result	Lab 6 result
Whole cloves	1	Positive	Negative	Positive	Positive	Negative	Positive
1:1,000	2	Positive	Positive	Positive	Positive	Positive	Positive
	3	Negative	Negative	Positive	Positive	Positive	Negative

Table 6: 1,000 CFU/25 g inoculum

Food	Sample Replicate	Lab 1 result	Lab 2 result	Lab 3 result	Lab 4 result	Lab 5 result	Lab 6 result
Whole cloves	1	Positive	Positive	Positive	Positive	Positive	Positive
1:1,000	2	Positive	Positive	Positive	Positive	Positive	Positive
	3	Positive	Positive	Positive	Positive	Positive	Positive

A 25 gram sample size was used, though diluted to overcome matrix effects. The number of replicates per concentration was 18, the number positive at 10 CFU/g was 0, the number positive at 100 CFU/g was 13 and the number positive at 1,000 CFU/g was 18. These numbers are entered into the spreadsheet. Based on the number of replicates, the coverage factor  $k = 2.01$ . Therefore, the LOD 50% is estimated at 2.398 CFU/g, with a lower limit of 1.441 CFU/g and an upper limit of 3.991 CFU/g.