

RECOMMENDED MICROBIOLOGICAL METHODS

Preface

The accompanying list of microbiological methods consists of official methods that are commonly used for a wide variety of food and food products including spices; other official or recognized methods not on the list may be applicable.

It is important to note that some spices and herbs contain essential oils that are inhibitory to bacteria and fungi and may produce low counts on low dilution plates and moderately high counts on higher dilution plates. This can be attributed to the carryover of the antimicrobial substances with the inoculum. When testing samples of certain spices, prepare a sufficient number of serial dilutions to overcome this natural inhibitory effect and prevent the reporting of erroneous low counts. This is particularly important for cloves, allspice, cinnamon, mustard seed oregano, onion and garlic. Results are generally not reported from the first dilution plates and tubes for these seven products. Inhibition may also be noted with nutmeg, sage, rosemary, marjoram and others.

The antimicrobial effects of the essential oils must also be taken into account when analyzing a spice for the presence of *Salmonella* and other pathogens that require a pre-enrichment step. Increasing the conventional 1:10 sample/Trypticase Soy Broth (TSB) ratio is recommended for some products. For example, the FDA's Bacteriological Analytical Manual recommends a 1:1000 sample/broth ratio for allspice, cinnamon and oregano and a 1:1000 sample/broth ratio for cloves¹. The results of a study conducted by Josephine Pafumi indicate that a 1:1000 sample/broth is necessary for cloves, allspice, cinnamon, oregano and mustard seed to ensure the detection of *Salmonella*². To neutralize the inhibitory properties of onion and garlic, prepare a 1:10 sample/broth ratio with TSB containing 0.5% K₂SO₃.³ Seasoning blends containing a large portion of one of the above mentioned spices should be pre-enriched like the pure spice.

Footnotes:

1. FDA Bacteriological Analytical Manual (BAM) 8th Ed. (1995) and BAM 8th Ed., Revision A (1998). Published by AOAC International, Gaithersburg, MD.
2. Pafumi, J. (1986) Assessment of the Microbiological Quality of Spices and Herbs. *J. of Food Protection*, Vol. 49, No. 12, p. 958-963.
3. Andrew, W. A., D. Wagner and M. J. Roetting (1979) Detection of *Salmonella* in Onion and Garlic Powders: Collaborative Study, *JAOAC*, Vol. 62, No. 3.

Analytical Method

Reference

Specific Citation

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Laboratory Quality Assurance	Compendium ^a SM ^b AOAC Int'l ^c	Chapter 1 Section 9020 See Footnotes
Sample Preparation / Dilution	BAM ^d AOAC Int'l ^e Compendium	Chapter 1 Chapter 5 (<i>Salmonella</i> Analysis) 967.26 (<i>Salmonella</i> Analysis) Chapter 2 Chapter 52
Aerobic Plate Count	BAM AOAC Int'l Compendium	Chapter 3 (Conventional) 966.23 (Conventional) 990.12 (Petrifilm) Chapter 7
Bacterial Spore Count	Compendium	Chapter 22 (Mesophilic Aerobic Sporeformers) Chapter 23 (Mesophilic Anaerobic Sporeformers)
Total Coliforms	BAM AOAC Int'l Compendium	Chapter 4 (MPN) 991.14 (Petrifilm) 992.30 (ColiComplete) Chapter 8
Fecal Coliforms	BAM Compendium	Chapter 4 Chapter 8
<i>E. coli</i>	BAM AOAC Int'l Compendium	Chapter 4 (MPN, LST/MUG or EC/MUG) 991.14 (Petrifilm) 992.30 (ColiComplete) Chapter 8
<i>E. coli</i> O157:H7	BAM AOAC Int'l Compendium	Chapter 4a 996.09 (VIP) 996.10 (EIA-Assurance) Chapter 35

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Yeast and Mold Count	BAM Compendium	Chapter 18 Chapter 20
<i>Bacillus cereus</i>	BAM AOAC Int'l Compendium	Chapter 14 980.31 Chapter 32
<i>Bacillus stearothermophilus</i>	Compendium	Chapter 25 (Thermophilic Flat Sour Sporeformers)
<i>Clostridium perfringens</i>	BAM AOAC Int'l Compendium	Chapter 16 976.30 Chapter 34
<i>Desulfotomaculum nigrificans</i> (Formerly <i>Clostridium nigrificans</i>)	AOAC Int'l Compendium	972.45 Chapter 27 (Sulfide Spoilage Sporeformers)
<i>Staphylococcus aureus</i>	BAM AOAC Int'l Compendium	Chapter 12 (MPN or Spread Plate) 987.09 (MPN) 975.55 (Spread Plate) 2001.05 (3M Petrifilm – Rapid <i>Staph. aureus</i> count plate) Chapter 39
<i>Salmonella spp.</i>	BAM AOAC Int'l Compendium	Chapter 5 989.14 (EIA-Tecra) 986.35 (EIA-Organon) 991.12 (ISO-GRID) 989.13 (1-2 Test) 992.11 (EIA-Assurance) 996.08 (VIDAS) 990.13 (GENE-TRAK) 989.15 (Q-Trol) 967.27 (Identification) 967.27 (Serological Tests) 978.24 (Biochem. Id.-API-20E) 989.12 (Biochem. Id.-MICRO-ID) Chapter 37

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<i>Shigella spp.</i>	BAM Compendium	Chapter 6 Chapter 38
Enterococci (Streptococci)	Compendium	Chapter 9
Lactobacilli	Compendium	Chapter 19
<i>Listeria monocytogenes</i>	BAM AOAC Int'l Compendium	Chapter 10 995.22 (EIA-Tecra) 994.03 (EIA-Organon) 996.14 (EIA-Assurance) 993.09 (GENE-TRAK) 997.03 (VIP) 992.18 (Biochem. Id.-Micro ID) 992.19 (Biochem. Id. - Vitek) Chapter 36
Environmental Monitoring	Compendium	Chapter 3
Gram stain	BAM	Reagents, R32

Footnotes:

- a - Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., 2001. Published by American Public Health Association, Washington, DC
- b - Standard Methods for the Examination of Water and Wastewater, 19th Ed., 1995. Published by American Public Health Association, Washington, DC. Section 9020.
- c - AOAC International Accreditation Criteria for Laboratories Performing Food Microbiological Testing
- d - FDA, Bacteriological Analytical Manual *Online*:
<http://www.cfsan.fda.gov/~ebam/bam-toc.html>
- e - Official Methods of Analysis of AOAC International, 17th Ed., 2000. Published by AOAC International, Gaithersburg, MD