NSF International Standard for Dietary Supplements — Dietary supplements

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7.3 Test methods for microbiological contaminants

7.3.1 Reference methods

Testing shall be performed based on the currently promulgated version of the United States Pharmacopeia (USP). With the exception of Pseudomonas, testing methods shall adhere to those described in USP <2021> Microbial Enumeration Tests – Nutritional and Dietary Supplements and USP <2022> Microbiological Procedures for Absence of Specified Microorganisms – Nutritional and Dietary Supplements. For Pseudomonas, testing methods shall adhere to those described in USP < 61> Microbiological Examination of Nonsterile Products: Microbial Test Limits.

NOTE – Methods that have been validated using established guidelines, such as those described by AOAC and USP, and have been demonstrated to yield equivalent or better results compared to the aforementioned USP methodologies may be substituted.

7.3.2 Preparatory Testing

Preparatory testing, as specified in the currently promulgated version of the United States Pharmacopeia (USP) shall be performed on all products. Certain products may themselves inhibit the multiplication of microorganisms that might be present, thus interfering with quantitative and qualitative microbiological assays detailed in section 7.3. Products shall be inoculated with the challenge microorganisms specified in USP <2021> and USP <2022>. For the quantitative assays, at least a 70% bioburden recovery compared to a control medium must be demonstrated. For the qualitative assays, the challenge organism must be recovered on the applicable selective media. If a product fails to meet the recovery limit, a suitable neutralizer (e.g. soy lecithin, 0.5%; or polysorbate 20, 4.0%) shall be added to the culture medium to neutralize inhibitory substances.

NOTE – In lieu of performing preparatory testing, a suitable neutralizer may be automatically added to the product and testing for the individual indicator organisms and pathogens may proceed as described in the following sections.

7.3.3 Total Aerobic Microbial Counts

Per the United States Pharmacopeia (USP), the Membrane Filtration Method or Plate method shall be used for products that are freely soluble. Moderately soluble and translucent products shall be processed via the Plate method. The Multiple-Tube Method shall be used for all other products. The media, diluent and incubation conditions specified by the USP shall be used.

7.3.4 Total Combined Molds and Yeasts Count

Per the United States Pharmacopeia (USP), the Membrane Filtration Method or Plate method shall be used for products that are freely soluble. Moderately soluble and translucent products shall be processed via the Plate method. The Multiple-Tube Method shall be used for all other products. The media, reagents and incubation conditions specified by the USP shall be used.

7.3.5 Enterobacteriaceae
Per the United States Pharmacopeia (USP), the sample shall be dissolved or suspended in Phosphate buffer or Fluid soybean casein digest medium and diluted to 100 mL with Fluid soybean casein digest medium. The suspension shall be pre-incubated and subsequently processed using the media, reagents and incubation conditions stated by USP.

7.3.6 *Salmonella spp*
Testing shall be performed based on the USP Test for the Absence of *Salmonella sp.* (USP <2022>).

7.3.7 *Escherichia coli*
7.3.7.1 Generic *Escherichia coli*
For finished products, testing shall be performed based on the qualitative USP Test for the Absence of *Escherichia coli* (USP <2022>).

7.3.7.2 Pathogenic *Escherichia coli*
If the presence of *E. coli* is confirmed, then testing shall be performed based the US FDA's Bacteriological Analytical Manual (BAM, Chapter 4A) to determine whether the product contains pathogenic *Escherichia coli*, including but not limited to 0157:H7.

7.3.8 *Staphylococcus aureus*
Testing shall be performed based on the USP Test for Absence of *S. aureus* (USP <2022>).

~~**7.3.9 *Clostridium species***~~
~~Testing shall be performed based on the USP Test for Absence of *Clostridium* (USP <2022>).~~

7.3.10 *Pseudomonas aeruginosa*
For semisolid or liquid products containing less than 25% alcohol v/v, testing shall be performed based on the USP <62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms.

7.3.1 Aflatoxins

Testing shall be performed based on the methods described in Chapter 49, Natural Toxins, pp 49-1 to 49-49 of the AOAC *Official Methods of Analysis*.

~~**7.3.2 Yeast and mold**~~

~~Testing shall be performed based on the USP Plate Count Method under Total Aerobic Microbial Count substituting Potato Dextrose Agar and altering the incubation time/temperature to 5-7 d at 25 °C (77 °F) or appropriately validated rapid testing procedure such as the Soleris system[4] "dilute to spec" protocol for yeasts and molds.~~

[4] ~~The user's attention is called to the possibility that compliance with this standard may require use of an invention covered by patent rights. By publication of this standard, no position is taken with respect to the validity of this claim or of any patent rights in connection therewith. The patent holder has, however, filed a statement of willingness to grant a license under these rights on reasonable and nondiscriminatory terms and conditions to applicants desiring to obtain such license. Patent holder for the Soleris system is Neogen, 620 Lesher Place Lansing, MI 48912, www.neogen.com.~~

7.3.3 Bacteria – total aerobic count

Testing shall be performed based on the USP Total Aerobic Microbial Count or appropriately validated rapid testing procedure such as the Soleris[29] system protocol for Total Viable Count.

7.3.4 Enterobacteriaceae

Testing shall be performed based on the USP Total Aerobic Microbial Count substituting m-ENDO agar as the agar medium or appropriately validated rapid testing procedure such as the Soleris system "dilute to spec" protocol for Enterobacteriaceae.

7.3.5 *Salmonella sp*

Testing shall be performed based on the USP Test for *Salmonella sp*.

7.3.6 *Escherichia coli*

7.3.6.1 Generic *Escherichia coli*

For finished products, testing shall be performed based on the qualitative USP Test for *Escherichia coli*. For raw materials, testing shall be performed based on a quantitative method adapted from the Enterobacteriaceae method (7.3.4). One to five colonies exhibiting a green metallic sheen shall be confirmed as generic *Escherichia coli* using the Conventional Method for coliforms, fecal coliforms, and *E. coli* presented in the USFDA Bacteriological Analytical Manual, Chapter 4.

7.3.6.2 Pathogenic *Escherichia coli*

If the presence of *Escherichia coli* is confirmed, then testing shall be performed based on the USFDA Bacteriological Analytical Manual in Chapter 4A to determine whether the colonies are pathogenic *Escherichia coli*, including but not limited to 0157:H7.

7.3.7 *Staphylococcus aureus*

Testing shall be performed based on the USP Test for *S. aureus*.

7.3.8 *Pseudomonas aeruginosa*

Testing shall be performed based on the USP Test for *P. aeruginosa*.

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