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⟨2023⟩ MICROBIOLOGICAL ATTRIBUTES OF NONSTERILE NUTRITIONAL AND DIETARY SUPPLEMENTS

INTRODUCTION

The raw materials, pharmaceutical ingredients, and active ingredients used in the manufacture of nutritional and dietary articles may range from chemically synthesized vitamins to plant extracts and animal byproducts, and these ingredients are typically not sterile. Considerable experience has accrued with these highly refined plant- and animal-derived pharmaceutical ingredients, such as microcrystalline cellulose, modified starch, lactose, and magnesium stearate, and their microbiological attributes are well established. Botanicals may be microbiologically contaminated at any point during cultivation, harvesting, processing, packing, and distribution. Major sources of microbial contamination are associated with human or animal feces used as plant manure; contaminated irrigation water and/or process water; and poor worker hygiene and sanitation practices during harvesting, sorting, processing, packaging, and transportation. Furthermore, it is essential that microbiological contamination be minimized during the manufacture of nonsterile dietary supplements. To achieve this, Good Manufacturing Practices are employed and adequate microbiological specifications are established.

Microbiological process control, control of the bioburden of raw materials, and control of the manufacturing process to minimize cross-contamination are necessary to guarantee acceptable microbial quality in the final dosage forms. Because nonaqueous or dry dosage forms do not support microbial growth because of low water activity, the microbial quality of such articles is a function of the microorganisms introduced through ingredients or during processing. In addition to considering the intended use of the product, the frequency of microbial testing for the finished nonsterile dietary supplement would be a function of the historical microbial testing database of that product, knowledge of the manufacturing processes, the susceptibility of the formulation to microbial proliferation, and the demonstrated effectiveness of programs controlling the raw materials.

FORMULATION AND PROCESS DESIGN

From a microbiological perspective, the development of the formulation of nutritional or dietary supplements includes an evaluation of raw materials and their suppliers and the contribution made to the products by each ingredient and the manufacturing processes. Characterization of these elements allows the adequacy of the manufacturing process to be demonstrated. For example, if a product is formulated with an ingredient of botanical or animal origin known to possess a high, variable, or unpredictable level of microbiological contamination, it is necessary to ensure that the microbiological monitoring identifies ingredients that have an inappropriate bioburden level and that a premanufacturing process such as drying, extraction, heat treatment, irradiation, or gaseous sterilization treatment will inactivate or remove any objectionable contaminant possibly present.

However, the selected treatment technique should not have any adverse effects. The treatment of raw materials by irradiation and ethylene oxide may cause unwanted changes affecting the safety and efficacy of the raw material. For instance, when treated by ethylene oxide, crude extracts containing alkaloids have shown reduced contents of alkaloids. Dry heat treatment has been used for inactivation as well, but requires further evaluation because it may adversely affect stability and degradation of the raw material. With regard to the design of the manufacturing process, appropriate consideration should be given to the microbiological effect of wet granulation manufacturing processes. Wetting of a dry powder can result in increased levels of microorganisms if the granulation is stored prior to drying. However, it is recognized that the pressure and temperature associated with compression of tablets will decrease microbial counts. Antimicrobial activity is also achieved, especially with aqueous preparations, by the addition of chemicals that have known antimicrobial properties and that are compatible with the formulation.

However, antimicrobial preservation is not a substitute for Good Manufacturing Practices. A process has to be designed to minimize the microbiological population. Operating procedures, temperatures, and time limits, including holding times, are established to protect the product from microbiological contamination and growth. All processes have to be validated for their intended purposes. Moreover, in-process manufacturing and testing controls necessary for microbiological quality should be identified and implemented.

FACILITIES, EQUIPMENT, WATER, AND SANITIZATION

Facilities

The facilities, including the building and the heating, ventilation, and air-conditioning (HVAC) systems, should be designed to minimize microbiological contamination. The design of facilities used for the manufacture of supplements and their operating parameters should be documented, and the documentation should include, when appropriate, HVAC filter types, space pressure differentials, temperature, and relative humidity and air changes. Dry products processed in a dry environment do not possess a high potential for increased microbial

levels. However, some control is warranted to minimize microbiological and chemical contamination. Potentially problematic areas are those that utilize *Purified Water* for wet granulation, batching liquid products, and film-coating tablets, because water encourages microbial growth.

Equipment

Equipment used for the processing of semisolid and dry supplements should be designed to promote sanitary conditions, to be self-drying, and to be easy to clean. Dryers, ovens, wet granulation equipment, bulk tanks, and equipment for preparation of coating solutions are periodically evaluated to ensure that cleaning procedures are adequate.

Water

As one of the major components in nutritional and dietary supplement manufacturing processes, water deserves a special consideration in the microbiological control of these articles. It is a growth medium for a variety of microorganisms that present a threat to product quality, safety, preservation, and stability. Water may even act as a carrier of objectionable microorganisms. In view of this, water used in manufacturing is *Purified Water*. For the manufacture of raw materials, process water that meets specific microbiological objectives and U.S. Environmental Protection Agency National Drinking Water standards or equivalent European and Japanese standards may be used.

Cleaning and Sanitization

Detailed and specific cleaning and sanitization procedures should be evaluated, developed, and validated, with special attention given to product contact surfaces. Personnel should possess sufficient knowledge of these procedures.

SUPPLEMENT COMPONENTS

Raw materials, excipients, and active substances as components of nutritional and dietary supplements can be a primary source of microbiological contamination. Specifications should be developed and sampling plans and test procedures should be employed to guarantee the desired microbiological attributes of these materials. The nature and extent of microbiological testing should be based upon a knowledge of the material's origin, its manufacturing process, use, historical data, and experience. For instance, materials of animal or botanical origin that are not highly refined might require special, more frequent testing than synthetic products.

Since members of the family Enterobacteriaceae are a major component of the normal epiphytic and endophytic microflora (e.g., members of genera *Klebsiella, Enterobacter*, and *Erwinia*) and have been associated with the seeds, pods, roots, leaves, and stems of plants of economic importance, coliform or Enterobacteriaceae counts will not be an appropriate general microbiological criterion for botanicals. However, when it is considered advantageous, coliform or Enterobacteriaceae counts may be included in the individual monographs. Typically on new leaves, bacteria predominate in the microflora, while yeast and filamentous fungi succeed bacteria and become dominant late in the growing season. With dried botanicals, the bacterial population will tend to change from Gram-negative bacteria to Gram-positive spore formers and fungi. Refinement of botanicals from chopped or powdered plant material to powdered extracts using alcoholic, alkaline, acid hydro-alcoholic, or aqueous extracting materials will reduce the likelihood of vegetative microorganisms within the botanical material. The classification of botanical materials is contained in *Table 1*.

Table 1. Definitions of a Range of Botanical Materials

Botanical Preparation	Definition
Chopped or Powdered Botanicals	Hand-picked portions of the botanical (e.g., leaves, flowers, roots, tubers, etc.) that are air dried, chopped, flaked, sectioned, ground, or pulverized to the consistency of a powder.
Botanical Extracts	Extracts are solids or semisolid preparations of a botanical that are prepared by percolation, filtration, and concentration by evaporation of the percolate. The extracting material may be alcoholic, alkaline, acid hydro-alcoholic, or aqueous in nature. Typically, an extract is 4–10 times as strong as the original botanical. The extracts may be semisolids or dry powders termed powdered extracts.
Tinctures	Tinctures are solutions of botanical substances in alcohol obtained by extraction of the powdered, flaked, or sectioned botanical.
Infusions	Infusions are solutions of botanical principles obtained by soaking the powdered botanical in hot or cold water for a specified time and straining. Typically, infusions are 5% in strength.
Decoctions	Decoctions are solutions of botanicals prepared by boiling the material in water for at least 15 min and straining. Typically, decoctions are 5% in strength.

Botanical Preparation	Definition
Fluidextracts	A fluidextract is an alcoholic liquid extract made by percolation of a botanical so that 1 mL of the fluidextract represents 1 g of the botanical.
Botanicals to be treated with boiling water before use	Dried botanicals to which boiling water is added immediately prior to consumption.

MICROBIOLOGICAL TESTING

Frequency of Sampling and Testing

Microbiological attribute sampling and testing plans vary widely. In some cases, no sampling or testing is necessary; in other cases, periodic monitoring is warranted; and yet for some articles, each batch requires sampling and testing. The design of the sampling and testing plans and the kind of attributes examined depend on the application and the type of the product, the potential for contamination from components and processing, the growth promotion or inhibition properties of the formulation, and the target population for the supplement. For example, a powdered botanical may have highly variable microbiological attributes so that an incoming batch would be sampled and composite testing would not be advised, while a highly refined botanical extract may not require routine microbial testing. Similarly, products with a low water activity will not be susceptible to microbial growth during their shelf life provided they are protected from elevated humidity by their containers.

Microbial Enumeration Tests

See the *Introduction* under *Microbial Enumeration Tests—Nutritional and Dietary Supplements ⟨2021⟩*. These tests provide meaningful information regarding the microbiological acceptability of excipients, active substances, and nonsterile supplement formulations. If the individual monograph does not specify microbial enumeration limits, the guidance provided in this chapter is used. Acceptable general limits of microbial levels for raw materials, excipients, and botanical products are shown in *Table 2*; and those for raw materials, excipients, active ingredients, and other nonsterile finished articles that are nutritional supplements, but do not contain botanicals, are shown in *Table 3*.

Table 2. Recommended Microbial Limits for Botanical Ingredients and Products

Material	Recommended Microbial Limit Requirements (cfu/g or mL)
Dried or Powdered Botanicals	Total aerobic microbial count NMT 10^5
	Total combined yeasts and molds count NMT 10^3
	Bile-tolerant Gram-negative bacteria NMT 10^3
	Absence of *Salmonella* spp. and *E. coli* in 10 g
Powdered Botanical Extracts	Total aerobic microbial count NMT 10^4
	Total combined yeasts and molds count NMT 10^3
	Absence of *Salmonella* spp. and *E. coli* in 10 g
Tinctures	Total aerobic microbial count NMT 10^4
	Total combined yeasts and molds count NMT 10^3
Fluidextracts	Total aerobic microbial count NMT 10^4
	Total combined yeasts and molds count NMT 10^3
Infusions/Decoctions	Total aerobic microbial count NMT 10^2
	Total combined yeasts and molds count NMT 10
Nutritional Supplements with Botanicals	Total aerobic microbial count NMT 10^4

Material	Recommended Microbial Limit Requirements (cfu/g or mL)
	Total combined yeasts and molds count NMT 10^3
	Absence of *Salmonella* spp. and *E. coli* in 10 g
	Total aerobic microbial count NMT 10^6
	Total combined yeasts and molds count NMT 10^4
	Bile-tolerant Gram-negative bacteria NMT 10^2
Botanicals to be treated with boiling water before use	Absence of *E. coli* and *Salmonella* spp. in 10 g

Table 3. Recommended Microbial Limits for Dietary Supplement Ingredients and Products

Material	Recommended Microbial Limit Requirements (cfu/g or mL)
	Total aerobic microbial count NMT 10^3
	Total combined yeasts and molds count NMT 10^2
Other raw materials and dietary supplement ingredients	Absence of *E. coli* in 10 g
	Total aerobic microbial count NMT 10^3
	Total combined yeasts and molds count NMT 10^2
Nutritional supplements with synthetic or highly refined ingredients	Absence of *E. coli* in 10 g

Absence of Objectionable Microorganisms

See *Introduction* under *Microbiological Procedures for Absence of Specified Microorganisms—Nutritional and Dietary Supplements ⟨2022⟩*. Absence of one or more species of objectionable microorganisms is required in some individual monographs.

Test for Aflatoxins

Dietary and nutritional articles containing botanical products with a history of mycotoxin contamination are also typically tested for aflatoxins, especially if the material is obtained from roots or rhizomes. See *Articles of Botanical Origin ⟨561⟩* for the details of a test for aflatoxins. Where necessary, this test is included in the individual monograph.

Solid Oral Dosage Forms

Among all dosage forms, solid oral dosage forms present the lowest microbiological risk because of their method of manufacture, low water activity, and route of administration. When justified, reduced microbiological testing may be appropriate.

Other Concerns

The presence of some microorganisms in articles can be an indicator of processes that are not under microbiological control. For example, *Purified Water* used at some stage of the manufacture of these products might contain a typical flora of Gram-negative microorganisms. As with pharmaceutical products, inadequate processing of water and poor maintenance of water systems may result in the contamination of processed formulations by Gram-negative microorganisms.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
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