Introduction

This document summarizes important points from the NACMCF document to assist retail food operators and regulators to use the document more easily. This document provides practical guidance to retail food facility operators looking to submit a food product for a challenge study, as well as to retail food regulators looking for assistance in reviewing a challenge study for approval. This CFP guidance document will primarily focus on extended holding of food products at room temperature, and extended date marking beyond 7 days, as these are the challenge studies primarily seen at retail. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) Parameters for Determining Inoculated Pack/Challenge Study Protocols is the accepted reference for conducting and reviewing challenge studies. The NACMCF document is detailed and comprehensive but may be difficult for some end users to apply without more training. Laboratories conducting challenge studies should have a complete and working understanding of the NACMCF document.

Different parts of this CFP guidance document are applicable to different stakeholders. Much of the NACMCF document is intended for use by the laboratory conducting the challenge study, specifically sections 3.0 through 12.0. Retail food operators should familiarize themselves with sections 1.0 through 3.0, but they should also understand sections 8.0 and 10.0 as their input is required. Retail food safety regulators working for agencies who approve variances within a jurisdiction should be familiar with sections 10.0 and 11.0 as they, along with their respective expert food microbiological laboratory personnel, are the ones reviewing challenge studies for approval.

The section numbers referenced in the NACMCF document were maintained in this guidance document to provide ease of reference between this document and the original NACMCF document.

Definitions

(Note: These definitions were adapted from standard dictionary definitions, using the context of the NACMCF document, and were written by the CFP committee.)

Anaerobic environment: An environment where little or no free oxygen exists. Certain microorganisms, such as *Clostridium botulinum* (the organism that causes botulism), can grow in anaerobic environments.

Challenge test/study: Microbiological testing performed to determine if a particular food requires time and/or temperature control to prevent pathogenic bacterial growth.

Competitive microflora: Yeasts, molds, and/or bacteria naturally or normally present in a food that can alter the behavior of the pathogen of concern. Competitive microorganisms can come from starter cultures, excessive inoculation, or typical or atypical spoilage organisms present in the food or introduced during the study. A

challenge study food sample should be collected from fresh product (i.e. within the first 10% of its normal shelf-life).

Control limit: A maximum and/or minimum value needed to control a biological, chemical or physical factor to prevent, eliminate or reduce to an acceptable level the occurrence of a food safety hazard.

Gas permeability: The state or quality of a material that allows gases to pass through it.

Headspace volume: Headspace is the internal volume of a package that is not occupied by the product.

Inactivation: To make or render something not active; to disable or cause not to function.

Indigenous microflora: The naturally occurring microorganisms in food in its natural state.

Inoculate: Intentionally introducing microorganisms into food or other substrate to see the extent to which they will grow, decline or survive.

ISO/IEC: The International Organization for Standardization/ International Electrotechnical Commission; a joint technical committee that sets standards for lab testing and calibration.

Multi-component product: A product, such as a chocolate chip cookie or a pizza, composed of distinct ingredients with varying fat, water, salt, or other constituents. A component can shield other ingredients from lethality during processing or alter the environment, such as by adjusting water activity (A_w) or pH, to allow microbial growth not generally expected with the ingredient.

Pathogen: A microorganism, such as *Salmonella*, that can cause illness or disease.

Product variability: The difference between batches (lots) of food in terms of specific properties such as color, texture, pH, water activity, etc.

Sampling interval: The timeframe that determines how often measurements will be taken during a challenge study.

Spoilage organisms: Bacteria, yeasts, and molds, that when present in a food in high concentrations, causes food to spoil or become otherwise unfit for eating.

Starter culture: Bacteria yeasts or mold, deliberately used during food production to cause specific changes in a food (carbon dioxide production, acid production, etc.).

Surrogate organisms: A nonpathogenic microorganism with similar growth or inactivation characteristics to a pathogenic microorganism

Worst-case formulation: A worst-case food formulation should have acidity, moisture, salt, A_w, etc. at extreme values identified for the product variability that are closest to those optimal for pathogen growth.

NACMCF section commentary

As noted above, the section numbers referenced below refer to the original numbering in the NACMCF document and have been retained in this to provide easy cross-referencing between this CFP guidance document and the original NACMCF document. In some case numbers appear to be missing if a section of the NACMCF document is not referenced in this CFP guidance document.

1.0 Obtaining expert advice and identifying a laboratory

The study should[1] be designed, conducted and evaluated by expert food microbiologists with knowledge of food products, food pathogens, and statistics. Personnel performing the study should have a combination of education, such as a B.S. in Microbiology, evidence of knowledge of basic microbiological techniques, and at least 2 years of challenge study experience or supervision by a microbiologist with that expertise.

A laboratory selected for challenge testing should be able to demonstrate prior experience in conducting or validating challenge studies and should meet laboratory standards for capacity and capability. Certifications (such as *ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories*) help identify laboratories capable of testing, but don't necessarily qualify a laboratory to design and conduct challenge studies. To conduct challenge studies, labs should also have approval and capacity to handle the organism(s) of concern as well as ensure appropriate microbial strains are used.

[1] Note: The committee uses the word should instead of must throughout the document as there may be instances where a scientifically valid study does not have all required components in order to be valid.

3.0 Factors related to test product

3.1 Product preparation.

The test product should be prepared under conditions most conducive to growth or survival based on the intended conditions of use and expected product variability (i.e. worst-case formulation). This includes ensuring the product is at equilibrium for physical properties (water activity, moisture, temperature, and pH) and that it is inoculated in areas most likely to become contaminated and/or where organisms would grow. The critical physical properties should be at worst-case limits for the finished product.

Multi-component products may take longer to equilibrate and should be inoculated prior to equilibration. Studies to determine growth, inactivation or survival of a pathogen present due to recontamination should be inoculated after equilibration.

3.2 Product variability.

Knowledge of the product variability over several product lots is needed to determine the appropriate testing parameters for a challenge study. The greater the variability, the more samples of product should be evaluated to identify the worst-case limits. Wherever possible, food should be processed to mimic conditions used during commercial operations and be representative of normal production. Adjustments to acidity, moisture, salt, water activity, etc. should be made to test a “worst case scenario”.

3.3 Competitive microflora.

Inoculated product should contain typical levels of competitive microflora, including starter cultures, but take care not to introduce atypical spoilage microorganisms. The study should ensure that the product evaluated was obtained and inoculated within the first 10% of its shelf life; for example, a product with a 30-day shelf-life should have the sample obtained and inoculated within 3 days of production.

4.0 Target Organisms

4.1 Identifying Pathogens of Concern

Organism selection is an important part of study design. A qualified study designer will determine what organism(s) to select. The organism(s) chosen will depend on a variety of factors, including the food storage temperature, pH, and aw. For example, consider *Clostridium botulinum* as a selected organism when evaluating foods held in anaerobic environments.

There are tables included in the NACMCF document that discuss organism selection that should be used to determine the proper organism for the challenge study. These tables

are labeled as Table 2, and Appendix C [4] [5] of the original NACMCF document. Both tables should be used together to select the proper organism for test. Preliminary testing on product for pH and water activity may be needed to help select organism(s) of concern.

4.2 Surrogate Organisms

There are certain circumstances in challenge testing that allow for the use of non-pathogenic surrogate organisms. If surrogates are to be used, their choice should be justified and valid for the food and the process being tested. The use of surrogate organisms may be most helpful to reduce cost and risk in product formulation design prior to conducting the challenge study.

8.0 Storage Conditions

8.1 Packaging

Products should be testing using the same conditions used for commercial packaging, including packaging materials and the process used for actual packing of the product. Attributes to consider include gas permeability, headspace volume, vacuum levels, and headspace gas composition. The conditions of the environment for packaging should also match the environment for commercial packaging.

8.2 Storage and Shipping temperatures

Storage and shipping temperatures should take into consideration product temperature variation. Humidity should also be taken into consideration for these tests.

NACMCF recommends that refrigerated foods be tested at 44.6°F (7 °C) to account for expected consumer storage temperature in the United States but may also be tested at other temperatures for a better understanding of microbial growth patterns. If a product may be subject to variation of temperatures during its shelf life, the product should be tested using these temperature variations.

Products being tested to determine their safety at ambient temperature should be tested using the expected storage room temperatures (typically 24 to 35°C or 75.2 to 95°F).

Reference 9.0 Sample Considerations

9.1 Sampling

The number of samples analyzed at each time interval should be at least two and any studies should be replicated at least twice with different batches of product and inocula. The number of replications depends on the product and the inoculum.

10.0. Duration of study and sampling intervals

For study duration parameters based on product shelf life, see chart 10.1.

Chart 10.1

Product type	Proposed Shelf Life	Additional Safety Margin
Sold for Immediate Service	7 to 10 days	50%
Sold for Immediate Service	10 days to 3 months	25% to 50%*
Sold for Immediate Service	3 to 6 months	25%
Packaged for Retail Sale at the food establishment	Any	50%

* at the discretion of the study designer

Food packaged at the retail establishment should use the most conservative additional safety margin provided in the NACMCF document, which is an additional 50%. Since the NACMCF document does not provide information on safety margin beyond 6 months, it is recommended that the proposed shelf life for a packaged product be determined by the microbiologist conducting the study, and should be between 7 days and 6 months.

Samples, including controls, should be analyzed initially after inoculation (*or after a short equilibration period at the direction of the study designer*) and then at least five to seven times over the duration of the study. For longer-shelf-life products, it may be necessary to have more than seven sampling points.

A study may be terminated when growth of the target pathogen exceeds 1 log for two or more consecutive sampling intervals, except in the case of *S. aureus* or~~,~~ *B. cereus,* ~~*or C. perfringens*~~ where NACMCF recommends 3-log. Studies may also be terminated when gross spoilage occurs.

11.0. Interpreting test results

The results of a microbiological growth study must be interpreted and evaluated by an expert microbiologist who will consider all relevant factors and the thresholds in the chart below. Smaller increases may be significant depending upon the enumeration methods, number of samples and replicates used, and the variability among data points. The regulatory authority can use more restrictive pass/fail criteria for a specific challenge

study based on the intended use of the product and the target consumer population (i.e. highly susceptible population).

The Pass/Fail criteria for test pathogens are listed below.

Chart 11.1

Pathogen	Pass	Fail
C. botulinum	No toxin detected for the duration of the study.	Any toxin detected during the study.
S. aureus or~~,~~ *B. cereus* ~~or C. perfringens~~ (if applicable)	In lieu of toxin testing, less than 3-log CFU/g growth above the initial inoculum level across all replicates.	Equal to or greater than 3-log CFU/g growth above the initial inoculum level in any replicates.
All other pathogens	Less than 1-log CFU/g growth above the initial inoculum level across all replicates.	Equal to or greater than 1-log CFU/g growth above the initial inoculum level in any replicates.

***A product does not support pathogen growth if growth has not exceeded the initial inoculum level by the limits listed above throughout the intended shelf life of the product and across replicate trials.**

When publishing the final report, ensure that the lab specifically states that the challenge study was conducted following the NACMCF Protocols.

Computer Modeling

The use of computer modeling for product assessment and pathogen growth in the absence of any laboratory data is limited. Only experimentally validated models for the specific pathogen(s) of concern should be used. Modeling can usually be used in excluding specific organisms of concern from consideration in challenge studies, (e.g., modeling shows than one pathogen grows faster, so the slow grower is excluded from subsequent laboratory studies).

Reference Documents:

1. *FSIS Report, Establishment Guidance For the Selection of a Commercial or Private Microbiological Testing Laboratory - https://www.fsis.usda.gov/wps/wcm/connect/464a4827-0c9a-4268-8651-b417bb6bba51/Guidance-Selection-Commercial-Private-Microbiological-Testing-lab-062013.pdf?MOD=AJPERES*

2. *Evaluation and Definition of Potentially Hazardous Food - https://www.fda.gov/downloads/food/foodborneillnesscontaminants/ucm545171.pdf*

3. *Parameters for Determining Inoculation Pack/Challenge Study Protocols - https://www.fsis.usda.gov/wps/wcm/connect/3b52f9c0-0585-4c0a-abf2-b4fc89a9668c/NACMCF_Inoculated_Pack_2009F.pdf?MOD=AJPERES*