General Testing Method Recommendations for Mold Count (microscopic count of mold hyphae, Howard Mold Count) in Various Food Matrices on IA 99-46

Date: 12/18/2024

**Please note, this recommendation is intended to provide supplemental general information to private laboratories on methods of analysis and test portion sizes. This document does not outline all the analytical method or worksheet requirements for packages being submitted for FDA review. **

Please refer to the current FDA Laboratory Manual, Volume III, Section 7 for comprehensive information on private laboratory package requirements and the review process:
https://www.fda.gov/media/73540/download

General notes

In general, when testing food products for filth, a lab should not use microbiological methods intended to determine viable mold and yeast, such as methods found in the Bacteriological Analytical Manual (BAM, https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam).

Howard Mold Count (HMC) can be part of filth testing of various products, such as drupelet berries, canned tomatoes, tomato soup, frozen strawberries, fruit pastes, and others.

For certain food matrices, but not all, the HMC method indicates that a laboratory cyclone or pulper is needed for sample preparation. Currently, the FDA is not aware of a cyclone in the market that fulfills all the specifications listed in AOAC Official method 945.75B(g). It is recommended to use a laboratory cyclone or pulper with specifications as close as possible to those listed in AOAC 945.75B(g). FDA has evaluated the Robot Coupe C80 with the 57009 (1/64”/0.5 mm) perforated basket and found it to be an adequate replacement for the cyclone, as specified in AOAC 945.75B(g).

It is not recommended to use a blender/homogenizer or a sieve as a substitute for a laboratory cyclone or pulper, or in conjunction with a cyclone/pulper, because this may affect the length of the fungal filaments, which are critical for considering a microscopic field as positive or negative.

Additionally, it is essential that the person performing the HMC analysis demonstrates proficiency performing HMC. Proficiency includes being able to properly mount slides and accurately differentiate mold structures from plant structures that may appear similar to mold filaments. Please refer to AOAC Official Method 984.29 Howard Mold Counting, part A ‘Diagnostic Characteristics of Mold’ and part B ‘Determination’ for detailed explanations of this method. An understanding of the information in AOAC 984.29 is essential; for example, observation of Newton’s rings is a critical aspect of the method. Examples of other publicly available documents regarding the HMC method are listed in the reference section.

Note: The information herein may be updated in the future, and if so, it will be posted on the same website.

References:

U.S. Food and Drug Administration. (2022). ORA Laboratory Manual Volume IV: Laboratory Training, Section 4- Microanalytical and Filth Analysis. https://www.fda.gov/science-research/field-science-and-laboratories/field-science-laboratory-manual

Cichowicz, S. M. (1981). Chapter 12: Analytical Mycology. In J. R. Gorham (Ed.), FDA Technical Bulletin No. 1: Principles of Food Analysis for Filth, Decomposition, and Foreign Matter (Second ed., pp. 191-200). U.S. DHHS.

Schulze, A. E. (1977). Chapter 14: Mold as an Index of Decomposition or Insanitation. In J. R. Gorham (Ed.), FDA Technical Bulletin No. 2: Training Manual for Analytical Entomology in the Food Industry (pp. 112-123).

Recommendations for Specific Products

Testing Method Recommendations for Mold Count (microscopic count of mold hyphae, Howard Mold Count) in Whole Canned Tomatoes and Diced Canned Tomatoes

**Please note, this recommendation is intended to provide supplemental general information to private laboratories on methods of analysis and test portion sizes of canned tomato products. This document does not outline all the analytical method or worksheet requirements for packages being submitted for FDA review. **

Please refer to the current FDA Laboratory Manual, Volume III, Section 7 for comprehensive information on private laboratory package requirements and the review process:
https://www.fda.gov/media/73540/download

Product Information:
Whole canned tomatoes and diced canned tomatoes consist of a main component (whole or diced tomatoes) and a packing medium (tomato puree, juice). **The packing medium (tomato puree, juice) should be analyzed for mold hyphae using the HMC technique first, then proceed with analysis of mold hyphae in the main component (whole or diced tomatoes)**.

Analytical Protocol:
Samples should consist of six sub-samples, each with at least one pound, for official analysis.

Sample Methods:

- Tomatoes (Whole, Diced, or Crushed), Canned:
    - Perform AOAC 945.90(a) on packing medium (tomato puree, juice). It may be helpful to use a #8 sieve for crushed tomatoes.

Testing Method Recommendations for Mold Count (microscopic count of mold hyphae, Howard Mold Count) in Drupelet Berries

**Please note, this recommendation is intended to provide supplemental general information to private laboratories on methods of analysis and test portion sizes of drupelet berry products. This document does not outline all the analytical method or worksheet requirements for packages being submitted for FDA review. **

Please refer to the current FDA Laboratory Manual, Volume III, Section 7 for comprehensive information on private laboratory package requirements and the review process:
https://www.fda.gov/media/73540/download

Product Information:
Applicable to blackberries, loganberries, raspberries, and other drupelets; fresh, canned, and frozen.

Analytical Protocol:
Samples should consist of six sub-samples, each with at least one pound, for official analysis.

Sample Methods:

- Frozen with or without sugar:
    - Perform AOAC 955.47(a)
- Frozen in syrup, canned in syrup or water:
    - Perform AOAC 955.47(b)

Quality Assurance:
Laboratory must follow the methodology specified in the private laboratory package submission. Any method modifications or deviations to the cited method must be explained and validation must be documented.

> FDA does not endorse any private laboratory firms, nor requires specific methods to be used for Private Laboratory Analytical Packages (PLAPs). Information herein is provided as a courtesy, but private laboratories are not required to use them. The requirements state the method should be locally validated and should adequately identify and or quantitate the violative analyte(s). The information herein may also provide supplementary sampling, method information and/or sample preparation information to assist private laboratories who are analyzing products being held under Detention Without Physical Examination (DWPE) as part of an Import Alert to assist private laboratories with submitting scientifically sound PLAPs as testimony pursuant to FD&C Act section 801 and 21 CFR 1.94 or FD&C Act section 422(b) and 21 CFR 1.1107.