



**United States
Department of
Agriculture**

Marketing and
Regulatory
Programs

Agricultural
Marketing
Service

Fruit and
Vegetable
Programs

Processed
Products
Branch

File Code
135-A-8

September 1998

Technical Inspection Procedure

Mold Count

PREFACE

This instruction is designed for Processed Products Branch personnel of the United States Department of Agriculture (USDA). Its purpose is to give background information and guidelines to assist in the uniform application and interpretation of U.S. grade standards, other similar specifications, and special procedures.

The citation of any data, criteria, techniques, illustrations, copyrighted material, or pictorial representation accredited to private authorship is used with the permission of the individuals or sources cited. Unless a specific reference is cited, the information in this instruction has been compiled or developed from sources available to the public as well as from technical knowledge of USDA personnel.

Compliance with the guidelines in this instruction does not excuse failure to comply with the Federal Food, Drug, and Cosmetic Act or any other applicable Federal or State laws or regulations.

Except for official inspection aids or devices and color guides (or standards) produced under license of the USDA, the mention of any supplier, patented device, product, brand name, or equipment does not imply endorsement by the USDA over any other similar, or equally effective, material.

Information concerning inspection and grading services provided by this Branch may be obtained from:

Chief, Processed Products Branch
Fruit and Vegetable Programs, AMS
United States Department of Agriculture
P.O. Box 96456, Room 0726 - South Building
Washington, DC 20090-6456

Telephone: (202) 720-4693

Fax: (202) 690-1527

This issue of File Code 138-A-8, Technical Inspection Procedure, Mold Count supersedes the previous edition dated May 1978.

James R. Rodeheaver
Branch Chief

Distribution:A
Agriculture:Washington

TABLE OF CONTENTS

SECTION 1 - GENERAL INFORMATION

I.	CHARACTERISTICS OF MOLD	1.1
II.	CLASSIFICATION OF MOLD	1.2

SECTION 2 - HOWARD MOLD COUNTS

I.	HOWARD MOLD COUNTING METHOD	2.1
A.	The Howard Mold Counting Chamber	2.1
B.	Preparation of the Mount	2.2
1.	Preparation of the Sample	2.2
2.	Cleaning the Chamber	2.2
3.	Transferring Sample to Cell	2.3
4.	Placing The Cover Glass	2.4
5.	Examine the Prepared Mount Before Counting	2.5
C.	Standardization of the Microscope	2.6
D.	Illumination	2.6
E.	Focusing	2.6
F.	Counting Patterns	2.7
G.	Examination of the Field	2.8
H.	Use of Howard Method	2.8

SECTION 3 - INSPECTION / SAMPLING PROCEDURES

I.	POLICY	3.1
II.	INSPECTION PROCEDURE	3.1
A.	Products Examined	3.1
B.	Sampling Rate	3.1
1.	Lot Inspection	3.1
2.	On-line Inspection	3.2
3.	Special Agreement Lot Inspection Service	3.3
C.	Number of Fields to Examine	3.3
III.	ACCEPTANCE CRITERIA	3.4
A.	Lot Inspection	3.4
B.	On-line Inspection	3.5
IV.	CERTIFICATION	3.6

SECTION 1 - GENERAL INFORMATION

I. GENERAL CHARACTERISTICS OF MOLD

The plant kingdom is divided into four major groups. The simplest plants are placed in the group called the Thallophyta. These Thallophytes are divided into two subdivisions, Algae and Fungi.

Mold belongs in the Fungi group, and is characterized by growing in irregular masses without the differentiation of roots, stems, and leaves found in higher plants. Molds lack chlorophyll, and are unable to manufacture their own food. Molds must obtain organic food from sources external to themselves. Consequently, they grow from feeding on dead plants and animals (Saprophytes) or from feeding on living plants and animals (Parasites).

Molds can reproduce in two ways:

By means of a tiny specialized cell called a spore. This spore, in the same manner as a flower seed, swells and germinates into a plant under the right conditions; and

By means of vegetative multiplication in which "broken-off-bits" of the plant can grow and form new plants much like the rooting of a geranium stem.

A long tubular filament grows out of the germinating spore or the "broken-off-piece" much like a sprout grows out of a bean seed. This filament is called a hypha. As the hypha continues to grow it starts to branch and rebranch until a whole system or body of fine intertwining, branching hyphae is formed. This system or body is called the mycelium. The mycelium spreads numerous fine branches out over the surface or throughout the mass of material from which the mold takes its nourishment. The hyphae break down the plant cells upon which they feed. This is called decomposition or rot.

The individual hypha is visible only under a microscope. It is only when the mycelium has grown into a large mass that it is visible to the naked eye. Mold identification is largely a microscopic technique when determining compliance with the applicable Food and Drug Administration (FDA) applicable Food Defect Action Level, see File Code 172-A-2.

II. CLASSIFICATION OF MOLD

The hyphae in the mycelium eventually divide into two types with separate functions. These types can be classified as:

A. Vegetative Type

The vegetative hyphae are those which grow underneath the surface and serve to digest and absorb the material on which the mold is growing.

B. Fertile Type

The fertile hyphae grow out from the mycelium like branches of a plant; the spores (like seeds of a plant) grow on the ends of the branches.

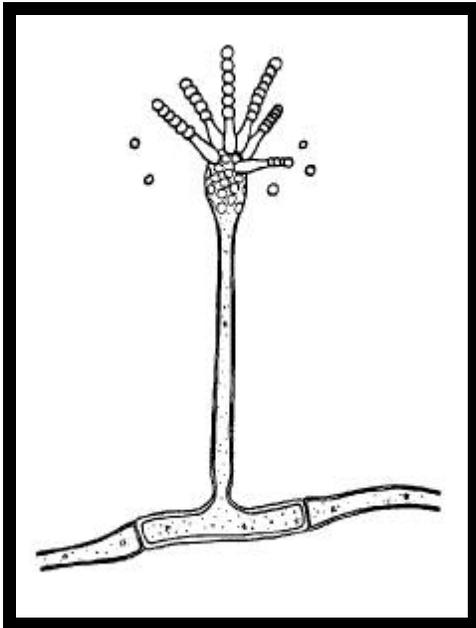
There are no distinctive differences between the vegetative hyphae of one species of mold and another. However, the spores or fruiting bodies of each species of mold have noticeable distinguishing features. Consequently, the classification of specific molds is based largely on the type spores produced from the mold's fertile hyphae.

Mold's fertile hyphae and spores are located on or above a product's surface. Therefore, these hyphae can be easily removed by washing and soaking the item that the mold is growing on. As a result, very little of this type of hypha remains on a processed fruit or vegetable by the time it is canned or frozen. On the other hand, submerged vegetative hyphae can be removed only by careful trimming and sorting.

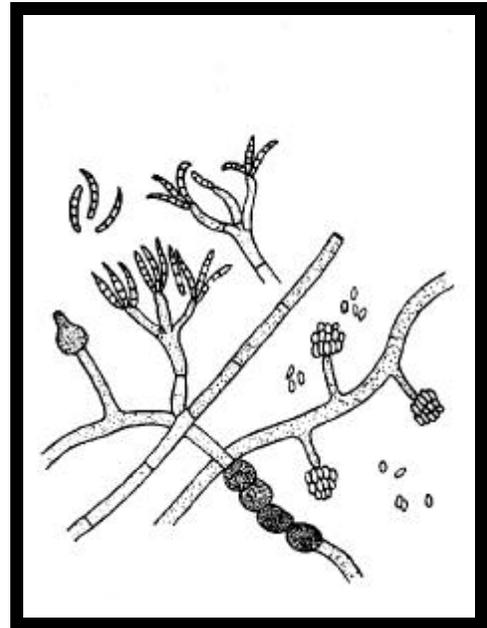
Since fruiting bodies of mold are removed from the raw material during normal sorting and processing procedures, seldom will one find hyphae at this stage in the finished processed product.

The inspector's main concern is the capability of distinguishing mold filaments from material that may resemble mold, but is not mold. Also, the inspector must be able to estimate the amount of mold present according to procedures detailed in this file code.

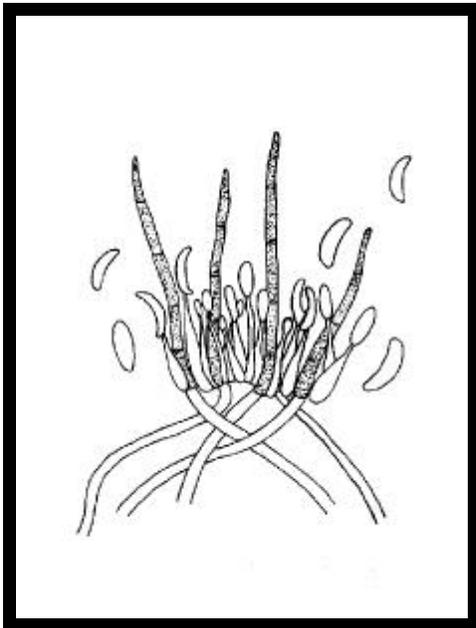
Examples of different types of spores are illustrated on the following pages.



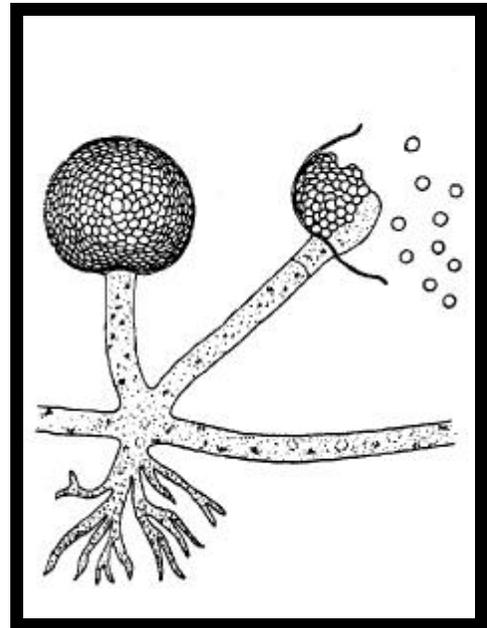
ASPERGILLUS



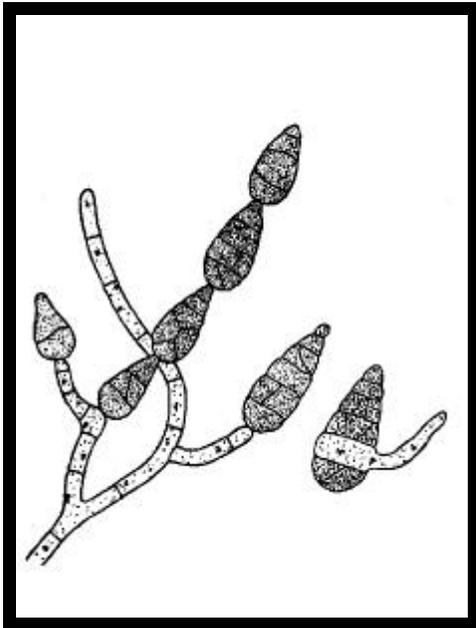
FUSARIUM



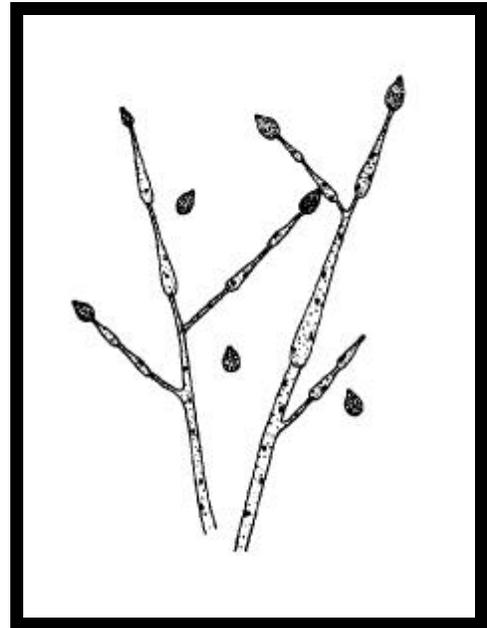
COLLETOTRICHUM



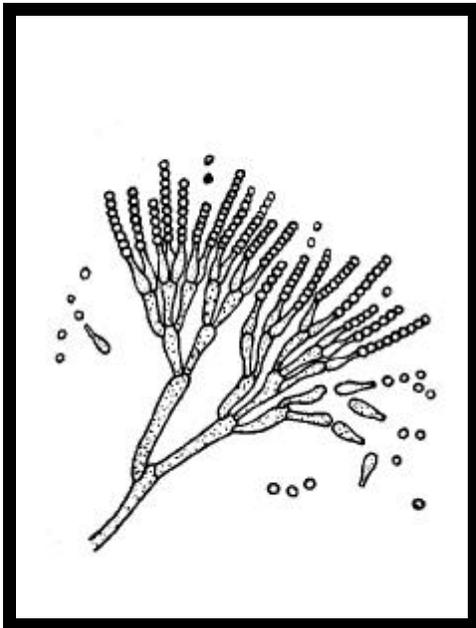
MUCOR & RHIZOPUS



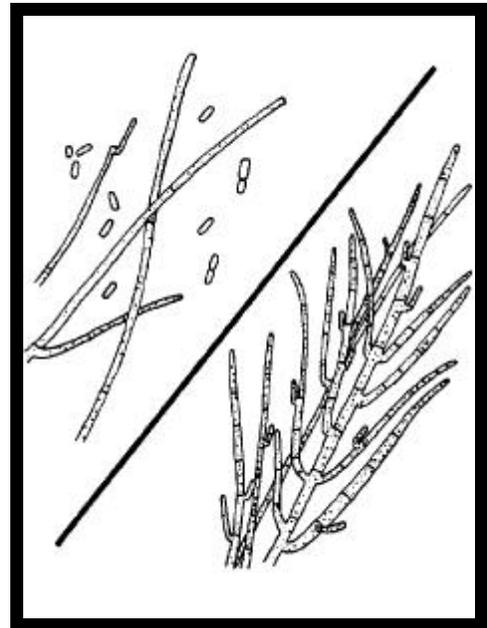
ALTERNARIA



PYHTOPHTHORA



PENICILLIUM



OOSPORA (OIDIUM)

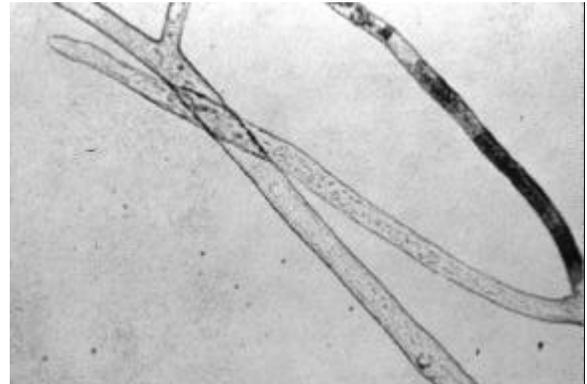
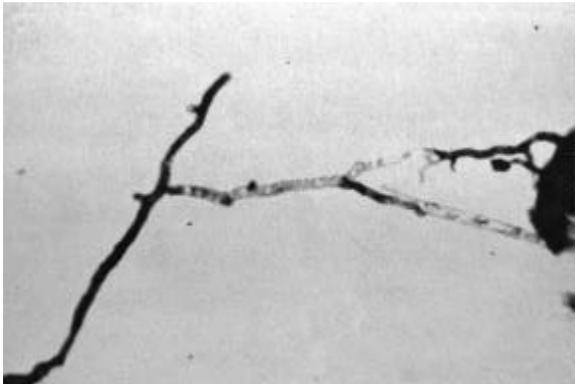
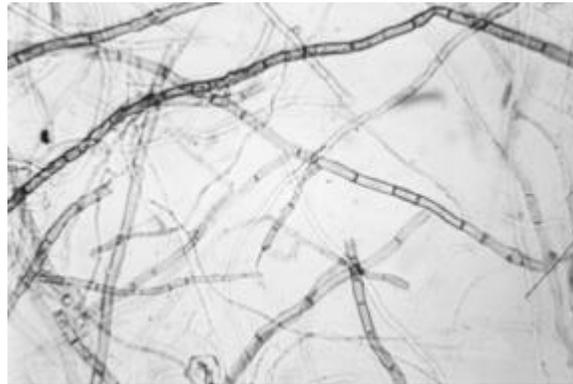
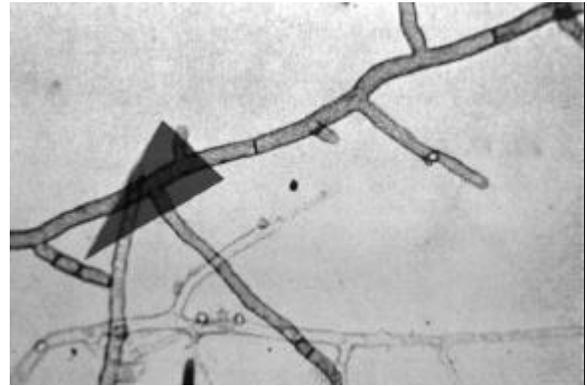
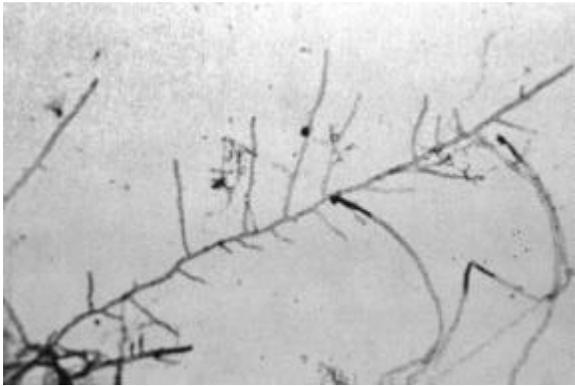


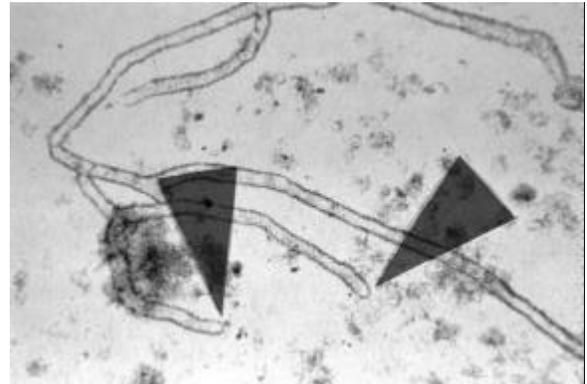
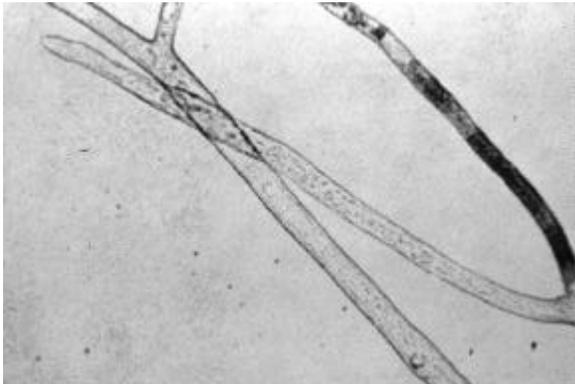
Illustration of blunt end of broken filament, granulation and branching.



Illustration of "broken" mold.



"Fine" mold hyphae illustrating parallel walls of even intensity, septa (cross walls), and branching.



Branched mold filaments showing granulation and parallel walls of even intensity and slightly rounded growing ends of branched mold hypha.

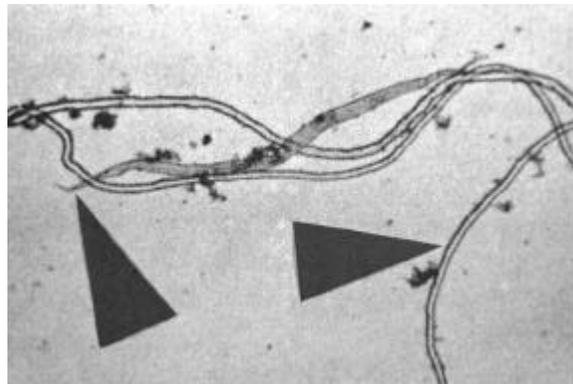


Illustration of structures easily confused with mold. Note frayed, pointed ends and walls of uneven intensity.

The following will aid in identifying mold filaments from material that is not mold. Only filaments which have at least one of the following six characteristics shall be classed as mold hyphae:

- | | | | |
|---|------|--|----------|
| 1. Parallel walls of even intensity with both ends definitely blunt. | This | | Not This |
| 2. Parallel walls of even intensity with characteristic branching. | | | |
| 3. Parallel walls of even intensity with characteristic granulation. | | | |
| 4. Parallel walls of even intensity with definite septation. | | | |
| 5. Occasionally encountered, parallel walls of even intensity with one end blunt and the other end rounded. | This | | Not This |
| 6. Occasionally encountered, slowly tapering walls of even intensity with one end with characteristic granulation or septation. | | | |

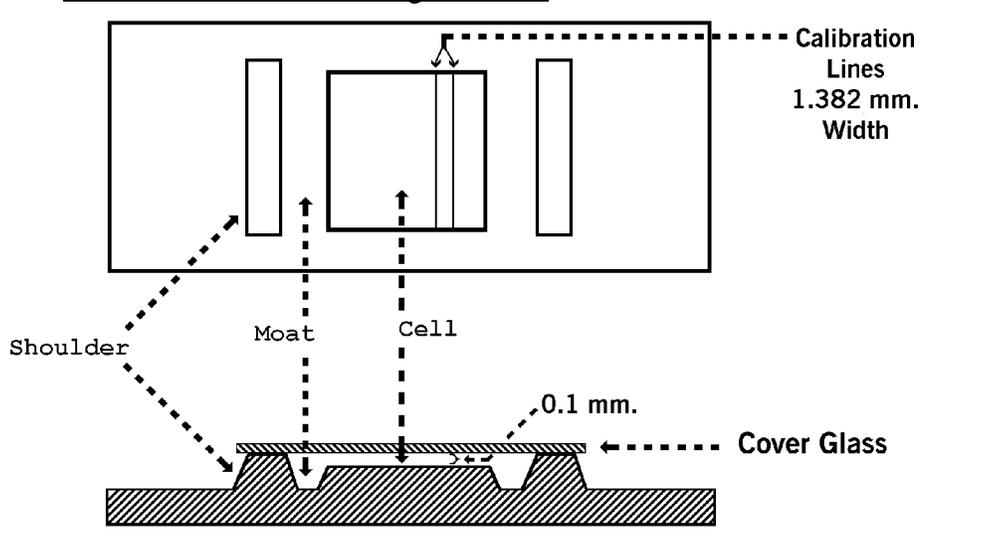
SECTION 2 - HOWARD MOLD COUNTS

I. HOWARD MOLD COUNTING METHOD

The Howard Mold counting technique is recognized internationally as a standard procedure for estimating the extent to which a product is contaminated with mold filaments. The "Howard Count" in turn can be related to the condition of the raw material as well as effectiveness of plant sanitation, particularly when such contamination is due to fungus type decay.

In performing a Howard Mold Count all details of sample preparation, slide preparation, counting technique, etc., must be carefully followed. Additionally, the inspector must have considerable training and supervision in the proper technique as well as proper identification of mold filaments. Otherwise, the results can be unreliable and erroneous conclusions may be drawn regarding the acceptability of the lot.

A. The Howard Mold Counting Chamber



$$\begin{aligned} \text{Area of circle} &= \pi r^2 = \frac{1}{4} \pi d^2 = 0.7854d^2 \\ \text{Area of Howard mold count microscopic field} &= \text{Diameter}^2 \times 0.7854 \\ &= 1.382^2 \text{ mm.} \times 0.7854 \\ &= 1.5 \text{ sq. mm.} \end{aligned}$$

$$\begin{aligned} \text{Volume of material in microscopic field} &= \text{Area} \times \text{Height} \\ &= 1.5 \text{ sq. mm.} \times 0.1 \text{ mm.} \\ &= 0.15 \text{ cu. mm.} \end{aligned}$$

B. Preparation of the Mount

1. Preparation of the Sample

Most products that require a mold count must be specially prepared before a sample of the product is transferred to the mold counting cell for actual mold counting. Refer to the applicable Instruction:

- a. Citrus Handbook - Florida Citrus Products.
- b. File Code 135-A-6, "Technical Inspection Procedures for Foreign Material in Canned Tomatoes and Tomato Products."
- c. File Code 135-A-5, "Foreign Material Canned and Frozen Berries and Related Products."
- d. File Code 135-A-9, "Preparation of Pineapple Products: Citrus Products and Fruit Nectars, Purees, and Pastes for the Howard Mold Counting Procedures."

2. Cleaning the Chamber

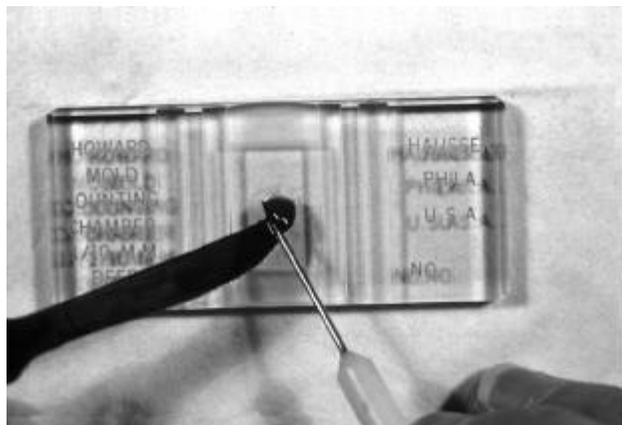
Wash the chamber and cover glass by dipping in a solution of soapy water, dilute ammonia, or alcohol. Dry with a lint free cloth.

Test for cleanliness by placing the cover glass in position and pressing it firmly against the shoulders of the slide. Then hold the chamber at an angle so that light is reflected from the cover glass. If a rainbow in a concentric circle appears between the shoulders and the cover glass, the chamber is considered to be clean. The rainbow effect is called "Newton's Rings." It is caused by interference between light beams reflected at the juncture of the two clean glass surfaces.

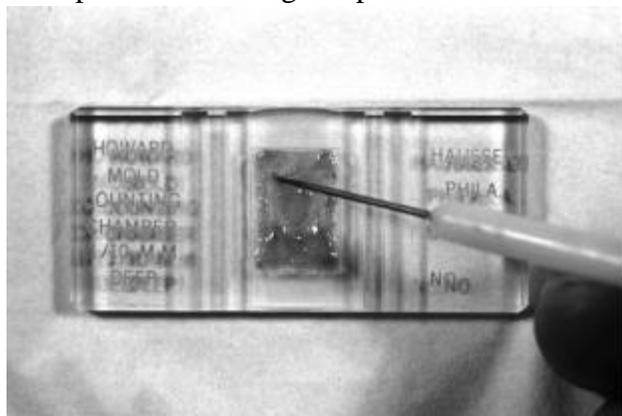
Newer Howard Mold counting chambers are constructed whereby the shoulders of the chamber contain a teflon coating. When the shoulders of the chamber contain this material "Newton's Rings" will not appear between the shoulders and the cover glass.

3. Transferring Sample to Cell

Dip the blade of a clean scalpel into the prepared sample with a scooping motion. Pick up a drop of the sample only large enough to cover the cell to the edge without squeezing an excessive amount into the moat when the cover glass is positioned. Spread the drop evenly over the surface of the cell. If the proper amount is not transferred, the slide should be cleaned and another transfer made. **DO NOT** add more sample to that already on the cell nor scrape off a part of that already there. By practice the inspector will become adept.



Step 1. Transferring sample to Howard cell.



Step 2. Pre-spreading sample on Howard cell.

4. Placing The Cover Glass

Position the cover glass at approximately a 45 degree angle with one edge resting on the shoulders of the chamber or suspend the glass entirely above the cell, then lower the glass in place. Practice will be needed in this technique. Lowering the glass too fast will splash the material onto the shoulder of the slide; and lowering too slowly will result in uneven distribution of the sample material on the cell.



Step 3. Positioning cover glass on slide shoulders.



Step 4. Lowering cover glass into place.

5. Examine the Prepared Mount Before Counting

The test material examined under each microscopic field is a very small amount (1/250 of a drop). Therefore, a well prepared slide is essential for uniform counting and comparable results. Before counting the mount, check for:

- a. Newton's Rings: Do not count the mount unless the rings appear (not applicable when the shoulders contain a teflon coating). The rings are an assurance there is uniform thickness of the test material on the cell.
- b. Uneven Distribution: Do not count a mount where there is visible evidence of uneven distribution of the insoluble material on the cell. When this happens there will be an uneven distribution of mold if it is present in the test material.
- c. Test Material In Moat or on Shoulders: Do not count the mount when there is visible evidence of material on the shoulders or an excessive amount in the moat because the cover glass will not fit properly and an uneven distribution of insoluble material and mold will result.
- d. Air Bubbles: A field with air bubbles may be counted as positive, but because of insufficient sample, it must never be recorded as negative and so must be skipped. Too many fields must not be skipped as all sections of the mount won't be represented.

C. Standardization of the Microscope

The microscope field must be checked against calibration marks etched on the mold counting slide before counting begins. The calibration marks are usually in the form of a circle or two parallel lines etched on one shoulder or on the cell portion of the slide. When properly adjusted, the field of vision will fall just within the outside lines of the calibration device. The proper drop-in micrometer disk inserted in the ocular of the microscope can be used to divide the field into squares. Each side of each square equals 1/6 of the diameter of the field. This calibration device is used in measuring the length of the mold filaments (see paragraph G. of this section). The field of vision will then measure 1.382 mm. as specified in the official method. Monocular microscopes are standardized by the optical companies. Monocular microscopes may be standardized by manipulating the draw tube.

D. Illumination

Proper illumination is very important. When different types of mold are present, it is often necessary to change the intensity of the light while the sample is being counted. This adjustment should be made only at the light source by addition or removal of filters, by manipulation of the iris diaphragm on the lamp, or by use of a rheostat. Too much light will conceal fine mold and too little light will not penetrate a mass of insoluble material sufficiently to reveal mold.

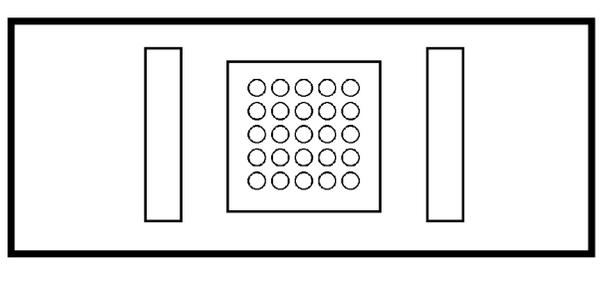
E. Focusing

Damage is often done to the objective of the microscope or the counting chamber by improper focusing technique. To prevent this, proceed as follows:

1. Place the slide in position on the stage of the microscope;
2. View both the slide and the objective of the microscope simultaneously;
3. **SLOWLY** bring the objective down until just before it touches the cover glass of the slide; and
4. Place the eye on the eye piece and bring the object into view by focusing the microscope tube **UPWARD**.

F. Counting Patterns

The Association of Official Analytical Chemists (AOAC) handbook that specifies the method of analysis requires an examination of at least 25 fields per mount taken in such a manner as to be representative of all sections of that mount. One way to get a good representation is by skipping every other field as the mount is moved vertically so the entire cell will be covered in an examination. This will cover the entire cell in the examination of 25 fields. A 25-field count on each mount is recommended. See example:



When counting fields in a slide it is important not to lose place of the sequence, as it could result in recounting a field. To prevent this, always count from left to right in the same manner as you read. This permits the work to become automatic and makes any interruption less confusing.

It is essential that the fields be selected systematically and that the slide is never moved to include or exclude mold filaments.

G. Examination of the Field

Observe each field in the counting pattern, noting the presence (positive) or absence (negative) of mold filaments and recording as positive when aggregate length of not more than three filaments exceed 1/6 of the diameter of the field.

It will save time to give each field a quick "once over." If there is enough mold in the field to make it positive, record it. If there is not enough mold, then all parts of the field should be carefully examined. Careful examination includes varying the intensity of the light occasionally and continuous use of the fine adjustment on the microscope. In some cases, a 200X magnification may be necessary to positively identify mold filaments observed in the standard magnification. Then return to 100X for classifying length and routine counting.

H. Use of Howard Method

The Howard Method requires a minimum of 25 fields per mount and 50 fields per subsample.

Counting 50 fields may be used in lieu of "USDA Mold Count Procedure" (see Section 3), provided;

1. It is requested by the applicant, prior to the inspection of a lot(s); and
2. Acceptance or rejection of the lot is based on this pre-determined method.

The Average Percent Mold is Calculated by:

$$\frac{\text{Total Number of Positive Fields}}{\text{Total Number of Fields Examined}} \times 100 = \text{Average}$$

SECTION 3 - SAMPLING / INSPECTION PROCEDURES

I. POLICY

The procedure in this manual must be used for inspection of processed fruits and vegetables which require acceptance for specified lot average mold limits.

II. INSPECTION PROCEDURE

A. Products Examined

Examine only those products listed in FDA's Food Defect Action Levels publication that have a specific mold defect action level, see File Code 172-A-2.

B. Sampling Rate

1. Lot Inspection

When the number of sample units examined for quality is: Examine the following number of subsamples for mold:

3	-----	1
6	-----	2
13	-----	3
21	-----	4
29	-----	5

2. On-line Inspection

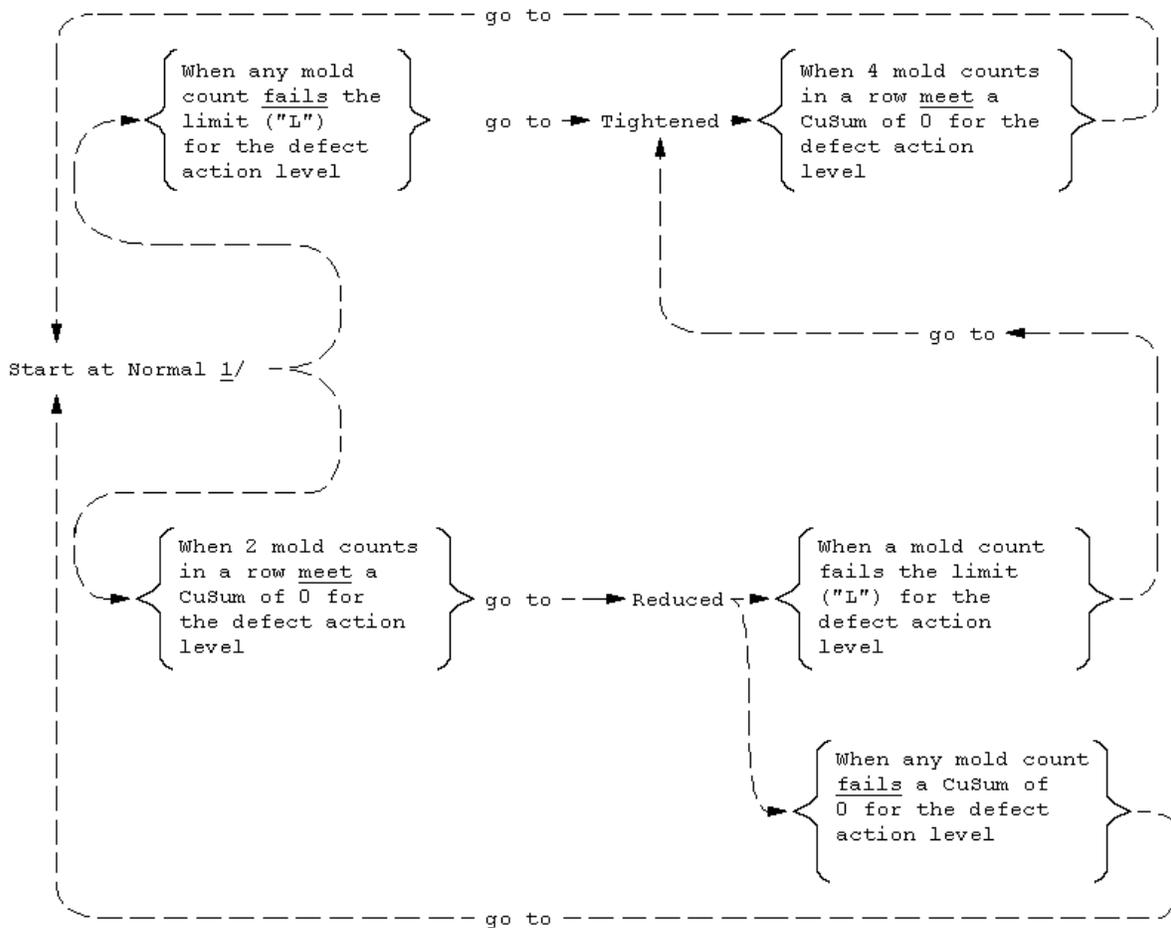
(a) Examine production at the following minimum rates:

Normal - 1 mold count every 4 hours.

Reduced - 1 mold count every 8 hours.

Tightened - 1 mold count every 2 hours.

(b) The rates may be switched as follows:



1/ Refer to "How to Compute CuSum Values" see File Code 120-A-6, (defectives are the positive fields).

3. Special Agreement Lot Inspection Service

When approval is granted by the Regional Director, the on-line sampling rate may be used for mold counting of products covered by Special Agreement Lot Inspection Service. This type of service provides for inspection of all products of a single type or can size. The basis for granting the on-line rate is that the processor's reliability and production history is established through a series of continuous inspection lots and consecutive code marks. The applicant must provide the area office with the coding system that will be used.

Example: When two mold counts in a row meet CuSum of 0 for the defect action level, the sampling rate for mold counts may be changed to the reduced rate, the equivalent of the one mold count for each eight hours of production, with a minimum of one count from each shift or production period.

C. Number of Fields to Examine

Examine 25 fields from each subsample selected for mold counting.

III. Acceptance Criteria

A. Lot Inspection

1. Accept the lot when the cumulative number of positive fields does not exceed the acceptance number for that defect action level and total fields counted.
2. Reinspect rejected lots, portions of lots, codes, subcodes, barrels, drums, pallets, and totes only by the "Howard Mold Count Method," page 2.8 of this manual.

Sample Size	Defect Action Level - Average Percent												
	5	10	12	15	20	25	30	35	40	45	50	55	60
	Cumulative Positive Fields												
25	2	4	5	5	7	8	10	12	13	14	15	16	18
50	3	6	8	9	12	15	18	22	23	26	28	31	33
75	4	8	10	12	16	21	25	31	33	37	41	44	48
100	5	11	13	15	21	27	32	41	43	48	53	58	63
125	6	13	16	18	25	32	39	50	52	59	65	71	78
150	7	15	18	21	30	38	46	59	62	70	77	85	92
175	8	17	21	24	34	44	53	68	71	80	89	98	107
200	9	19	23	27	38	49	60	77	81	91	101	112	122
AQL <u>1/</u>	2.5	6.5	8.5	10.0	15.0	20.0	25.0	33.0	35.0	40.0	45.0	50.0	55.0

1/ AQL expressed as percent defective.

B. On-line Inspection

1. Accept the production when CuSum does not exceed the Acceptance Limit ("L") for the defect action level. Acceptance is required to comply with step 5 of the CuSum procedure outlined in File Code 120-A-6.
2. Reinspect rejected production only as an appeal lot inspection.
3. If rejected production is packed so that containers are separately identifiable (totes, barrels, etc.), the cutoff point between acceptable mold limits and unacceptable mold limits may be established by counting back from the point where production exceeded the limit to the last acceptable production. **DO NOT USE CUSUM.** Use the "Howard Mold Count Method." Each container is a separate lot.

Defect Action Level - Average Percent													
	5	10	12	15	20	25	30	35	40	45	50	55	60
Maximum Number of Positive Fields													
S	0	1	0	1	0	1	2	2	1	2	2	1	1
T	1	2	3	3	5	6	7	9	10	11	12	14	15
L	1	3	2	3	2	3	4	5	4	5	5	3	4
AQL <u>1/</u>	2.5	6.5	8.5	10.0	15.0	20.0	25.0	33.0	35.0	40.0	45.0	50.0	55.0

1/ AQL expressed as percent defective.

Use File Code 120-A-6 as the manual for guidance and interpretation of "S," "T," and "L" values. For CuSum mold counting, "Sample Unit Tolerance" (T) is the allowable number of positive fields in any subsample (25 fields). The "Acceptance Limit" (L) is the maximum allowable accumulation of positive fields exceeding the sample unit tolerance (T) in any subsample or any consecutive subsamples.

When the number of positive fields exceeds a CuSum of 0 for any defect action level (average percent), production is approaching a rejection level.

NOTE: When the number of positive fields exceeds the Sample Unit Tolerance (T), positive fields would exceed an acceptance limit of 0.

IV. CERTIFICATION

Refer to File Code 165-A-1 for appropriate certification statements. Mold count results are certified only when requested by the applicant.