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**TECHNICAL INSPECTION
PROCEDURES FOR
FOREIGN MATERIAL IN
CANNED TOMATOES
AND TOMATO PRODUCTS**

PREFACE

This manual is designed for Processed Products Branch personnel of the U.S. 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.

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DROSOPHILA EGG AN LARVA DETERMINATION

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This File Code Supersedes the following:

File Code 135-A-6, dated June 1978

Revised pages 7 and 8, December 1981

Revised pages 9, April 1979

Branch Notice 2018, dated August 1973 - "Technical Inspection Procedures for Foreign Material in Canned Tomatoes and Tomato Products."

FILE CODE 135-A-6

MOLD COUNT

I. SAMPLING RATE/FREQUENCY

Follow the procedure in File Code 135-A-8 (Mold Count pages 3.1, 3.2, and 3.3).

II. SAMPLE PREPARATION

Prepare the sample for mold counting as outlined in Table I of this manual.

TABLE I

| | | | | | | |
|-------------|---|--------------|--|---|--|---------------------|
| Product | Canned Tomatoes and Stewed Tomatoes all styles | Tomato Juice | Tomato Sauce | Tomato Paste | Tomato Puree | Conc.. Tomato Juice |
| Preparation | Drain product; use packing medium without dilution. <u>2/</u> | | | Dilute with water to 7.9% to 8.8% Tomato Soluble Solids | | |
| Product | Tomato Catsup | Chili Sauce | Pizza Sauce, Crushed Tomatoes, Salsa, & other similar products | Tomato Powder | Specialty Products (canned dried beans in tomato sauce, pork and beans, soup, spaghetti, etc.) | |
| Preparation | Mix 1:1 with stabilizer <u>1/</u> | | Dilute with water to 6% total solids <u>1/</u> | Mix 17 g with 200ml of water | Follow AOAC procedure. | |

1 See the additional special procedure in this manual.

2/ In the event of an appeal inspection of a failing lot for mold count, follow the AOAC procedure for Canned Whole Tomatoes.

II. SAMPLE PREPARATION

A. Canned Tomatoes-all styles (any packing medium), Stewed Tomatoes, Tomatoes and Okra, and Other Similar Products.

Drain the tomatoes on a number 2 circular sieve for two minutes, except that the number 8 circular sieve is used for sliced and diced styles of canned and stewed tomatoes. Make a Howard Mold count of packing medium.

Under in-plant inspection, at the discretion of the inspector, the point of sampling for mold counting may be any of the following:

1. Common source (single or multiple products; or
2. Filler bowl (topping medium); or
3. Warehouse sample.

B. Tomato Juice and Tomato Sauce.

Under in-plant inspection, at the discretion of the inspector, the point of sampling for mold counting may be any of the following:

1. Common source (single product or multiple products); or
2. Warehouse sample (individual containers).

C. Tomato Paste, Tomato Puree, and Concentrated Tomato Juice.

$$\frac{(8.5)(100)}{\text{Tomato Soluble Solids (\% of Paste, Puree; or Juice)}} = \text{Grams of Product}$$

Mix the grams of product indicated above with water until 100g is reached (product + water). Use this sample unit for color, defects, and mold counting.

Under in-plant inspection, the point of sampling may be as in B.

- D. Chili Sauce, Pizza Sauce, Crushed Tomatoes, Catsup, Salsa, and other similar products.

Under in-plant inspection, the point of sampling may be as B.

Follow the pulping guidelines given below, except for catsup.

Pulping guidelines:

Use the entire contents of the individual container;
or a suitable aliquot from a well mixed container; and

Pass the product through an approved laboratory pulper to remove seeds and large particles. Follow File Code 135-A-5 as guidelines for use of the pulper.

- E. Tomato Catsup and Chili Sauce.

(1) Use one of the following stabilizing solutions for counting mold (1:1 dilution).

1. 0.5% Sodium Carboxyl Methyl Cellulose (CMC). A source of supply is:

Hercules, Inc.
Cellulose & Protein Products Dept.
910 Market Street
Wilmington, Delaware 19899

Specify: "Cellulose Gum CMC-7 HSF"

Preparation of CMC:

Place 500 ml of boiling water in high speed blender. With blender running, slowly add 2.5 g of the cellulose gum (CMC) and blend for one minute. Treat the solution with heat or vacuum to remove air bubbles. After the solution has cooled, add 2 ml of formaldehyde for each 100 ml of solution as a preservative, mix well, and store in a closed container. Larger volumes of CMC solution may be prepared ahead of the season. Label the solution:

"POISON FORMALIN ADDED." Small "poison decals may be available from your local pharmacy.

2. 3% to 5% Pectin Solution

Follow the same procedure as the CMC solution procedure, except, use 15 g to 25 g of pectin.

3. 1% Algin Solution

Follow the same procedure as the CMC solution procedure, except, use 5 g of Algin.

CAUTION: Check all stabilizer solutions for mold growth if formaldehyde has not been added or the solution has been stored from the previous season.

(2) Use the following method for mixing the product with the stabilizing solution:

1. Place 50 ml of the stabilizing solution in a 100 ml graduated cylinder;
2. Add 50 ml of well mixed product sample to the cylinder by displacement. Drop catsup or chili sauce directly into the solution and mix thoroughly in the cylinder. If the product is allowed to run down the side of the cylinder, the graduations are difficult to read; and
3. Transfer the mixture to a suitable container (beaker) and determine the mold count. Use 1 drop of caprylic alcohol if air bubbles are a problem.

Alternate method:

1. Tare a beaker;
2. Add 50 ml of the stabilizing solution and weigh (use this weight for any subsequent samples); and
3. Add 50 ml of product (use this weight for any subsequent samples; and
4. Mix thoroughly and determine the mold count. Use 1 drop of caprylic alcohol if air bubbles are a problem.

CAUTION: If the total solids of the product should change, such as from 33% to 29%, make a new determination for the weight of 50 ml of product.

I. SAMPLING RATE/FREQUENCY

A. Lot inspection

1. Domestic - Examine at the rate of one analysis per inspection lot.

If any eggs or larva are found, examine the lot at the rate indicated in Table II of File Code 172-A-1.

2. Imported - Examine at the rate indicated in Table III of File Code 172-A-1.

B. On Line Inspection

Examine at the rate of one analysis every 24 hours, for each product. A common source beyond which no further product contamination will occur may be used.

If any eggs or larva are found, examine at the rate of one analysis every 8 hours, with a minimum of one analysis for each shift or period, on each lot.

Return to the lower rate when no eggs or larva have been found for the past 24 hours of production.

C. Special Agreement Lot Inspection

With the approval of the regional director, the on-line sampling rate may be used for egg and larva determination of products covered by Special Agreement Lot Inspection Service. These types of agreements provide 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. Applicant must provide the officer-in-charge with the coding system that will be used.

II. SAMPLE SIZE

Use the amount of sample for drosophila egg and larva counting as outlined in

Table II of this manual.

| | | | | | |
|-------------------|--------------------|-------------------|----------------------------|--------------|-------------|
| Product <u>4/</u> | Canned Tomatoes | Tomato Juice | Conc. Tomato Juice | Tomato Sauce | |
| Sample Size | 500 g <u>1/</u> | 100g <u>2/</u> | | | |
| Product <u>4/</u> | Tomato Puree | Tomato Paste | Tomato Catsup <u>3/</u> | Chili Sauce | Pizza Sauce |
| Sample Size | 100g <u>2/</u> | | | | |

- 1/ Multiples of 100g may be used. From cumulative results, determine the average number of eggs and larva for each 500g increment.
- 2/ If the filtered trapping contains excessive pulp the amount of product may be reduced to 75g, or 25g. Calculate and record the equivalent number of eggs and larva per 100g of product.
- 3/ Egg and larva determination are not routinely performed on Tomato Catsup, unless requested by the applicant.
- 4/ See the additional special procedure in this manual.

A. Preparation

1. Canned Tomatoes (any packing medium), Stewed Tomatoes,

Tomatoes and Okra, and Other Similar Products

Under in-plant inspection, at the discretion of the inspector, the point of sampling for egg and larva may be any of the following:

- a. Common source (single product or multiple products); or
- b. Filler bowl (topping medium; or
- c. Warehouse sample (tomatoes and "associated" drained packing medium from individual containers.

Prepare the sample as follows:

- a. Place 500g aliquot (tomatoes and "associated" packing medium) on an 8-mesh sieve. Rest the sieve in the inside cone of a large funnel.
- b. Rinse the container that was used to transfer the aliquot to the funnel. Add the rinse water to the funnel.
- c. Wash the tomatoes with a fine spray of warm water (125 degrees F). Use as little water as possible.
- d. Repeat and completely wash all material on the screen.
- e. Use the procedure outlined under "Method of Extraction." (Page 9, section IV).
 - a. If the volume of washings and juice is 1/2 the volume of a No. 10 can, one separatory funnel is probably sufficient to make good, clean separation of pulpy material in the juice.
 - b. If the volume of washings and juice exceeds 1/2 the volume of a No. 10 can or if multiples of 500g aliquots are used, more than 1 separatory funnel will be needed.

Reminder: Do not select the 500g aliquot from diluted packing medium. Select the 500g aliquot and add washings to it. not vice versa.

2. All Products Other Than Canned Tomatoes and Other Similar Products.

Use the product directly from the container without dilution.

For pizza sauce and chili sauce, wash the aliquot with warm water (125 degrees F) through a 10-mesh sieve. The residue left on the screen is transferred to a black bottom pan and examined for the presence of larva. File Code 135-A-5 may be used as a guideline for this procedure. The drained portion is transferred to a separatory funnel for extraction.

Under in-plant inspection, at the discretion of the inspector it is permissible to use common source sampling. Whenever a common source sample exceeds the defect action level it would be necessary to hold all product affected, and sample individually the finished products to determine the extent of contamination.

III. PREPARATION OF PECTOLYTIC ENZYME SOLUTION.

1. Prepare fresh daily.
2. Add 1 level teaspoon of pectolytic enzyme (Pectinol AC) to 300 ml of warm water (125 degrees F). Stir well for 2 minutes.

NOTE: High temperatures cause rapid denaturation of pectinol enzymes. Optimum temperature for pectinol depectinization is 120 - 130 degrees F.

3. Let settle, then pour off the clear solution. Discard the sediment.
4. Make larger amounts of solution by using multiples of the proportion given under (2).
5. Optionally, use 0.5g of potassium oxalate in the above solution to break up gelation of material in the separatory funnel. It is especially helpful with catsup.

SOURCE OF SUPPLY

Pectinol AC

Genencor Inc.
Baron Steuben Place
Corning, New York 14831
Tel: 800-847-5311
ATT: Gloria Mathews

IV> METHOD OF EXTRACTION

1. Identify each sample and its corresponding separatory funnel.

2. Wash the sample into the separatory funnel and rinse the transfer container.
3. Add about 15 ml of pectolytic enzyme solution, shake, and let stand 15 minutes or longer.
4. Add about 30 ml of white gasoline. Stopper, invert, release the pressure and shake vigorously for one minute. Release the pressure as necessary.

CAUTION: White gasoline should be stored in a safety can.

5. Add warm water (125 degrees F) and bring the level of the contents to the wide portion of the funnel. Swirl and invert the funnel several times.
6. Let stand 15 minutes or longer and drain about 20 ml into a 400 ml beaker. Filter and examine as directed in 7 and 8. If no eggs or larva are found, discontinue subsequent drainings.
7. Filter the trapped liquid in the 400 ml beaker through black 10XX bolting cloth with vacuum (aspirator or vacuum pump). Ruled black filter paper may be used as a substitute for the bolting cloth. Discard the filter paper after each use.
8. Examine the bolting cloth or filter paper under 20X magnification.
9. If one or more egg or larva are found, repeat swirling, standing for 15 minutes, and draining 2 more times. The last time, remove about 200 ml of the bottom liquid (a total of 240 ml).
10. Filter and examine, compare results with the tolerance in File Code 172-A-1.

LIGHT FILTH DETERMINATION

I. GENERAL

There are no defect action levels for light filth (oil flotation Wildman trap flask) for tomatoes or tomato products in File Code 172-A-1, Inspection Procedures for Foreign Material. Make this determination only at the request of the applicant. Report the results to the applicant, but do not use them for official action.

II. PREPARATION OF SAMPLE

A 100g sub-sample is to be taken from each of a minimum of three containers of product in preparing an aliquot for the light filth extraction. If possible the aliquot should be taken from containers that have the same code marks.

- A. Canned Tomatoes, Canned Dried Beans in Tomato Sauce, Pork and Beans, and Other Products Having Both Solid and Liquid Components.

In most cases, the inspector will find it necessary to drain more than three containers to obtain the required sub-sample of 100g. It is important that as many containers as necessary be used to composite the proper aliquot.

- B. Chili Sauce, Pizza Sauce, and Other Similar Products Containing Seed and/or Peel.

In products of this type do not remove seeds and peel from the aliquot. It is important that the product be thoroughly mixed before obtaining the sub-sample.

- C. Tomato Catsup, Tomato Juice, Tomato Puree, and All Other Comminuted Tomato Products.

Thoroughly mix total contents of container.

In the case of concentrated tomato juice, tomato puree and tomato paste, take an amount of product from the 300g sub-sample which, diluted with water to 5.5 percent natural tomato soluble solids, will give the specified aliquot of 200g.

Following are the steps in obtaining the required aliquot for these concentrated products:

1. Determine the percent natural tomato soluble solids of the 300g sub-sample to the nearest 0.5 percent. (See Methods of Analyses for Solids Determination).
2. Find on Table III of this section the percent natural tomato soluble solids of the sub-sample. Adjacent to this figure is the number of grams of product to be taken from 300g sub-sample to obtain the required aliquot.

For Example:

If the percent of natural tomato soluble solids of the 300g sub-sample is 14.5 percent the required amount of puree to be taken from this sub-sample for light filth determination is 75.9g. (For products in the puree range of natural tomato soluble solids, the aliquot may be weighed to the nearest gram; in the above example, 76g.)

or

If the percent of natural tomato soluble solids of the 300g sub-sample is 32.0 percent the required amount of paste to be taken from this sub-sample for light filth determination is 34.4g. (For products in the paste range of natural tomato soluble solids, the aliquot must be weighed to the nearest 0.1g.)

This aliquot may then be directly washed into the clean Wildman trap flask as outlined in the extraction procedure.

For any given percent of N.T.S.S. of the sub-sample, the adjacent gram figure represents the amount of product which, when brought up to 200 grams with water, will yield an aliquot of 200 grams at 5.5 percent N.T.S.S.

TABLE III

| % N.T.S.S. of Sub-Sample | Grams of Product | % N.T.S.S. of Sub-Sample | Grams of Product | % N.T.S.S. of Sub-Sample | Grams of Product |
|--------------------------|------------------|--------------------------|------------------|--------------------------|------------------|
| 8.5 | 129.4 | 26.0 | | 42.3 | 25.3 |
| 9.0 | 122.2 | 26.5 | 41.5 | 44.0 | 25.0 |
| 9.5 | 115.8 | 27.0 | 40.7 | 44.5 | 24.7 |
| 10.0 | 110.0 | 27.5 | 40.0 | 45.0 | 24.5 |
| 10.5 | 104.8 | 28.0 | 39.3 | 45.5 | 24.2 |

| | | | | | |
|------|-------|------|------|------|------|
| 11.0 | 100.0 | 28.5 | 38.6 | 46.0 | 23.9 |
| 11.5 | 95.6 | 29.0 | 37.9 | 46.5 | 23.6 |
| 12.0 | 91.7 | 29.5 | 37.3 | 47.0 | 23.4 |
| 12.5 | 88.0 | 30.0 | 36.7 | 47.5 | 23.2 |
| 13.0 | 84.6 | 30.5 | 36.0 | 48.0 | 22.9 |
| 13.5 | 81.5 | 31.0 | 35.5 | 48.5 | 22.6 |
| 14.0 | 78.6 | 31.5 | 34.9 | 49.0 | 22.4 |
| 14.5 | 75.9 | 32.0 | 34.4 | 49.5 | 22.2 |
| 15.0 | 73.3 | 32.5 | 33.8 | 50.0 | 22.0 |
| 15.5 | 70.9 | 33.0 | 33.3 | 50.5 | 21.8 |
| 16.0 | 68.7 | 33.5 | 32.8 | 51.0 | 21.5 |
| 16.5 | 66.7 | 34.0 | 32.4 | 51.5 | 21.4 |
| 17.0 | 64.7 | 34.5 | 31.9 | 52.0 | 21.2 |
| 17.5 | 62.9 | 35.0 | 31.4 | 52.5 | 21.0 |
| 18.0 | 61.1 | 35.5 | 31.0 | 53.0 | 20.7 |
| 18.5 | 59.5 | 36.0 | 30.5 | 53.5 | 20.5 |
| 19.0 | 57.9 | 36.5 | 30.2 | 54.0 | 20.3 |
| 19.5 | 56.4 | 37.0 | 29.7 | 54.5 | 20.2 |
| 20.0 | 55.0 | 37.5 | 29.3 | 55.0 | 20.0 |
| 20.5 | 53.6 | 38.0 | 28.9 | 55.5 | 19.8 |
| 21.0 | 52.3 | 38.5 | 28.6 | 56.0 | 19.6 |
| 21.5 | 51.1 | 39.0 | 28.2 | 56.5 | 19.4 |
| 22.0 | 50.0 | 39.5 | 27.9 | 57.0 | 19.2 |
| 22.5 | 48.9 | 40.0 | 27.5 | 57.5 | 19.1 |
| 23.0 | 47.8 | 40.5 | 27.1 | 58.0 | 19.0 |
| 23.5 | 46.8 | 41.0 | 26.9 | 58.5 | 18.8 |
| 24.0 | 45.8 | 41.5 | 26.5 | 59.0 | 18.6 |
| 24.5 | 44.9 | 42.0 | 26.2 | 59.5 | 18.5 |
| 25.0 | 44.0 | 42.5 | 25.8 | 60.0 | 18.3 |
| 25.5 | 43.2 | 43.0 | 25.6 | 60.5 | 18.2 |

NTSS = Natural Tomato Soluble Solids

III SAMPLE SIZE

The aliquots to use in making light filth extraction from products containing tomatoes or

tomato products are as follows:

| PRODUCT | ALIQUOT SIZE |
|---|---|
| Tomato Paste | 200 g. at 5.5 percent natural tomato soluble solids |
| Tomato Puree | 200 g. at 5.5 percent natural tomato soluble solids |
| Tomato Juice | 200 g. |
| Tomato Catsup | 80 g. |
| Chile Sauce | 100 g. |
| Tomato Sauce | 120 g. |
| Pizza Sauce (all styles) | 120 g. |
| Spaghetti Sauce (all styles except dehydrated) | 100 g. |
| Tomato Soup | 200 g. |
| Canned Tomatoes | 300 g. |
| Canned Tomatoes and Puree | 200 g. |
| Canned Stewed Tomatoes | 300 g. |
| Canned Tomatoes and Okra | 300 g. |
| Canned Salad Tomatoes | 300 g. |
| Canned Okra and Tomatoes | 300 g. |
| Spaghetti with Sauce, Pork and Beans and Similar Specialty Products | 300 g. |

IV EXTRACTION OF SAMPLE

A. Equipment and Materials

Wildman trap flask (a 2-liter, thin walled, narrow-mouthed Erlenmeyer flask, with a plunger about 18" in length made of a brass rod to which is attached a rubber disk 55 mm. in diameter).

400 ml. beakers. Do not use polyethylene containers.

Wash bottles.

Stirring rods.

Adequate vacuum filtration apparatus with a Hirsch funnel containing a fine screen (about 30 mesh).

Petri dish or thick glass plate.

Greenough type, stereoscopic, binocular microscope with magnification of specimen parts.

Spotlight of sufficient illumination to permit ready identification of specimen parts.

Sharp probe.

Light mineral oil.

White, unleaded gasoline.

Deaerated, room temperature (about 75 degrees F) and hot (about 140 degrees F.) water.

Filter paper (Whatman No. 4, 7 cm. in diameter, ruled), or the equivalent.

A 25 percent glycerine and water solution.

Magnetic stirring equipment (optional).

1. Clean the extraction flask thoroughly so that water will drain from it completely rather than clinging in drops. To do this, scrub the flask with a brush and soap or detergent and rinse it several times. Repeat as necessary for thorough washing.
2. Wash the sample into the cleaned flask, using 600 - 800 ml. of water (about 75 degrees F.).
3. Add 36 ml. of light mineral oil.
4. Tip the flask and move the plunger disk below the surface of the liquid in quick, short up and down strokes, drawing the oil into the sample. Avoid beating in any air. Mix for two minutes.

OR

An alternate method for mixing oil into the sample is as follows:

- (a) Weigh sample in a 400 ml. beaker and add a small amount of water to facilitate stirring.
 - (b) Place beaker on a magnetic type stirrer and begin mixing. Once a vortex is formed, the oil is poured into the sample.
 - (c) Mix the sample two minutes. It is important that the speed of the magnet be regulated so that air is not incorporated. After mixing the sample it may be washed directly into the Wildman trap flask.
5. Fill the flask slowly almost to the neck with water (75 degrees F), pouring down the side of the flask to avoid getting air into the sample. Deaerated water may be obtained by allowing tap water to settle in a flask until the air bubbles rise or by vacuum method, using aspirator apparatus attached to sink faucet.

See Inspection Aid No. 51 "Filter Tank Aid Used for Performing Light Filth Extraction."

6. Allow the flask to stand about 10 minutes, stirring only during the first 5 minutes.
7. Scrape the drops of oil from the sides of the flask with the plunger, avoiding excessive disturbance of the suspended oil layer.

8. Add hot water (140 degrees F) slowly to bring the oil into the neck of the flask.
9. Raise the plunger disk slowly into position below the oil and pull it up gently but firmly. Wash the oil carefully off the plunger rod into the flask with hot water from a wash bottle.
10. Decant the trapped oil layer into a beaker. Wash out the neck of the flask with hot water while the flask is still held at an inclined angle over the beaker.
11. Return the flask to the upright position. Rinse the oil off the stirring rod with into the flask. Work the rubber disk loose with a stirring rod and lower plunger into the flask.
12. Allow the flask to stand 10 minutes, gently stirring occasionally. Scrape the side, allow to settle, bring the oil level into the neck with hot water. Trap and decant oil layer into beaker. Wash the neck of the flask thoroughly with hot water into the beaker. Repeat decanting as often as necessary to recover practically all of the oil.

If the trapped off oil-water portion is relatively free of tomato material it may be filtered immediately. If it contains considerable debris, or excessive pulp material, discard the contents of the flask, wash thoroughly, rinse and fill about 1/4 full with clean, hot deaerated water. Gently pour the trapped-off oil fraction into the flask and rinse beaker into flask. Carefully bring oil level into neck with hot water. Allow to stand 10 minutes. Repeat scraping, settling, and decant into the beaker. Repeat decanting to the beaker as often as necessary to recover practically all of the oil. In these final decantings, gasoline, followed by hot water, may be used to help rinse the plunger rod and the neck of the flask. The purpose of re-washing in the flask is to obtain a cleaner filter paper, but the procedure should not be performed more than once on any sample. The re-washing procedure is usually necessary when extracting light filth from tomato products to avoid excessive loss of time in searching the filter paper; extractions of some products are sufficiently free of pulp without washing.

13. Press a ruled wetted filter paper into the suction funnel and carefully pour the oil and washings (down a stirring rod) from the beaker onto the paper. Rinse out the beaker thoroughly with gasoline followed by hot water, pouring both onto the filter paper.
14. Remove the filter paper to a Petri dish or thick glass plate and moisten

with a 25 percent glycerine and water solution. Keep the filter paper moist, but not so wet as to float fragments.

C. Recording Results

Use a light strong enough to show all details on the filter paper as seen through the microscope. Using 30X magnification, count by probing with a needle over the whole surface of the paper, lane by lane. Do not count doubtful material. Mark the paper alongside each counted fragment with a Venus blue pencil for future checking and to avoid miscounts.

A magnification of 60 - 75 X is sometimes useful for examining and identifying questionable particles as light filth or not. After such identification, use the 30 X magnification.

Isolate, identify, and record all worm or insect fragments and rodent type hairs.

PROCEDURE FOR COUNTING ROT FRAGMENTS

I. DEFINITION

Rot Fragment - A particle of tomato cellular material with one or more mold filaments attached. (Some may appear as almost solid masses of mold).

II. SOLUTIONS

1. Crystal Violet Solution - Add 10g of the crystal violet dye into 100 ml of ethyl alcohol. Stir and let stand three minutes.
2. Stabilizer Solution - Pour 500 ml of boiling water into a blender. With blender running, add 2.5 g of stabilizer and 10 ml of formaldehyde. (See page 3 for the types of stabilizer that may be used).

III. PROCEDURE

1. Tomato Juice, Tomato Sauce, or Tomato Catsup: Weigh 10g and transfer with 100 ml of water to a 400 ml beaker.
2. Tomato Puree or Tomato Paste: Dilute to a refractive index of 1.3448 to 1.3454 at 20 degrees C. (approximately 8.0-9.0 percent NTSS). Weigh 5g of the diluted product and transfer with 100 ml of water to a 400 ml beaker.
3. Add 10 drops of crystal violet solution to the 400 ml beaker. Stir and let stand three minutes.
4. Add 200 ml of water to the 400 ml beaker.
5. Pour contents of the 400 ml beaker evenly over the surface of a three inch number 60 sieve.
6. Rinse with water (using wash bottle) the 400 ml beaker and pour rinsings evenly over the surface of the number 60 sieve.
7. Tilt the number 60 sieve to an approximate 30 degree angle and wash the tissue on the screen to the lower edge with water (using wash bottle).
8. Drain the tissue and transfer to a graduated tube (12x3 mm) with a spatula.

9. Rinse any remaining tissue on the screen to the lower edge with water from an eye dropper. Use the eye dropper to transfer the tissue and rinse water into the graduated tube.
10. Bring volume of water and tissue in the graduated tube up to 10 ml with water.
11. Bring volume of water and tissue in the graduated tube up to 20 ml with stabilizer solution.

12. Pipette two separate 0.5 ml portions from the graduated tube and spread evenly over the counting surface of two separate rot counting slides. Let the material flow slowly onto the slide to cover an area of approximately 6x2 centimeters.

NOTE: Use a 1 ml measuring pipette with the tip of the pipette cut off at the 1 ml mark.

13. Place cover glasses on counting slides.
14. Examine each slide under a wide field microscope using 35x to 40x power and transmitted light.
15. Count the number of rot fragments on each of the two slides. Add results and multiply by 2 (for the 10 gram sample), or multiply by 4 (for the 5 gram sample) $\frac{1}{2}$ to obtain number of rot fragments per gram of tomato product.

$\frac{1}{2}$ Reference Section III, 1 and 2.