METHODS OF ANALYSES

for

TOMATO PRODUCTS

Fly Egg and Maggot Determinations

FOR USE OF USDA PROCESSED FOODS INSPECTORS

UNITED STATES DEPARTMENT OF AGRICULTURE
CONSUMER AND MARKETING SERVICE
FRUIT AND VEGETABLE DIVISION
PROCESSED PRODUCTS STANDARDIZATION AND INSPECTION BRANCH

METHODS OF ANALYSES
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TOMATO PRODUCTS

FLY EGG AND MAGGOT DETERMINATION

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November 1967

These instructions with respect to Fly Egg and Maggot Determination in Tomato Products supersede:

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METHODS OF ANALYSES
for
TOMATO PRODUCTS

FLY EGG AND MAGGOT DETERMINATION

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PREFACE

These instructions are designed primarily for Processed Fruit and Vegetable Inspectors of the U. S. Department of Agriculture. They are not intended to be a comprehensive treatise on the subject but give background information and guide-lines to assist in the uniform application and interpretation of USDA grade standards and other similar specifications.

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INSTRUCTIONS FOR

FLY EGG AND MAGGOT DETERMINATION

I BACKGROUND AND INCIDENCE

Canned tomatoes and tomato products are particularly susceptible to contamination by eggs and maggots of the Drosophila fly -- often referred to as fruit fly, vinegar fly, or sometimes, gnat. Fly egg and/or maggot infestation has resulted in many Food and Drug seizures and other actions. It is one of the more serious contamination problems and one to which we must be ever alert.

The majority of eggs laid by adult Drosophila in the field are deposited in fresh cracks in the fruit principally between 6 to 8 A.M. and 4 to 8 P.M. when the light is not too strong nor the temperature too high or too low. The rate of egg deposition during these periods has been found to be 25 to 35 times the rate during the rest of the day. The high rate of deposition may be extended on overcast days.

Although some eggs may be deposited in growth cracks which occur while the fruit is still attached to the plant, the primary attractant for egg deposition is fresh cracks with free juice, resulting from picking and handling or weather changes. Nearly all of the eggs found on the fruit at the canning plant have been laid in the fresh cracks after the fruit is picked.

Some of the newer crack-resistant varieties of tomatoes have proven quite successful in decreasing the degree of fly egg deposition.

Other practices which are significant contributions toward controlling the fly egg problem are:

1) Use of clean, dry containers in the fields during harvesting operations;

2) Assurance that the containers (boxes or baskets) are not overfilled and that they are loaded and stacked on the truck in such a manner as to avoid breakage of the fruit during loading and in transit to the canning plant;

3) Certainty that no picked tomatoes are permitted to remain in the field overnight when flies are present;

4) Good sanitary practices and adequate washing and clean-up facilities in the plant; and

5) Effective spraying or dusting with insecticides which are formulated and applied in conformance with USDA specifications.

For a comprehensive discussion of the drosophila problem refer to the NCA bulletin on "Drosophila Control."

II IDENTIFICATION AND CLASSIFICATION

Fly eggs are tiny (approximately 0.25 mm. x 0.10 mm.) long-oval, symmetrical, yellow or whitish; covered with a transparent membrane; vinegar fly eggs have paired filaments near one end.

Maggots are long, tapering or baggy, yellow or whitish, symmetrical and faintly segmented, without legs; with dark mouth hooks at one end.

For the purpose of classification (in order to apply the guides which provide acceptance or rejection criteria) this type of infestation is considered under three (3) general categories:

1) Fly eggs.

2) Maggots 3 mm. or less in length.

3) Maggots over 3 mm. in length.

III PRODUCT GROUPINGS OR CLASSIFICATIONS

The nature of tomato products varies as does the preparation of sample and the number of fly egg and maggot counts which are performed routinely. The final extraction method is similar in all cases.

Processed tomato products are classified into these following general categories for purposes of relating similar items, preparing samples, and establishing minimum numbers of extractions.

A CANNED TOMATOES, and such related canned items as to include but are not limited to:

- Canned tomatoes with Puree
- Stewed tomatoes
- Canned tomatoes with Okra (or Okra with Tomatoes)
- Unpeeled tomatoes
- Diced, Sliced or Chunk Styles of Tomatoes

B COMMINUTED TOMATO PRODUCTS, whether canned or otherwise processed, including but not limited to:

- Tomato Puree (Tomato Pulp) and Concentrated Tomato Juice
- Tomato Paste
- Tomato Juice
- Tomato Catsup
- Tomato Sauce

C "SEEDED" TOMATO PRODUCTS, are those containing seeds, peel particles and condiments and which include but are not limited to:

- Pizza Sauce
- Chili Sauce

D SPECIALTY PRODUCTS, in which tomato products are an important ingredient, including but not limited to:

- Spaghetti with Tomato Sauce
- Pork and Beans with Tomato Sauce
- Bar-B-Que and Cocktail Sauces
- Tomato Soup
- Spaghetti Sauces
- Tomato Aspic

IV SAMPLING RATES

A LOT INSPECTION

The following charts indicate the normal and tightened sampling rates for canned tomatoes and tomato products when these stated conditions apply:

1) Normal rate -- is to be used when the individual sample units for fly egg and maggot determination contains no more than 1 maggot up to 2 mm or no maggots over 2 mm, or does not exceed 3/4ths of the limit specified for good commerical practice.

2) Tightened rate -- is to be used when any individual sample unit for fly egg and maggot determination * exceeds the limit(s) for the normal rate given above.

* Revised - February 1971

IV SAMPLING RATES (continued)

A LOT INSPECTION (continued)

CANNED TOMATOES

No. of Quality Samples as specified for single sampling plan in regulations	Normal	Tightened
3	1	2
6	2	3
13	3	5
21	4	6
29	5	7
38	6	9
48	7	11
60	8	13
Each increment, or portion thereof, of 3,000 cases in excess of maximum number of cases covered by 60 sample units	1	2

TABLE II

TOMATO PRODUCTS

No. of Quality Samples as specified for single sampling plan in regulations	Normal	Tightened
3	1	2
6	2	3
13	3	5
21	5	7
Each increment, or portion thereof, of 4,000 cases in excess of maximum number of cases covered by 21 sample units	1	2

Addition and correction - February 1971

IV SAMPLING RATES (Continued)

B INPLANT INSPECTION

* General

Inplant inspectors may determine fly egg counts on individual samples rather than composite sampling such as specified under lot inspection. Portions of production which fail the guide for good commerical practice will be considered an incomplete grade subject to further examination. Segregated portions of continuous production shall be examined on the tightened procedures for lot inspection.

Sampling Plans

There are three sampling plans for inplant inspection:

1) Normal

2) Reduced

3) Tightened

The sampling rate for the normal plan is one fly egg and maggot count every three hours for each source of continuous production.

\+ In most cases at the beginning of production the sampling rate will be on the normal plan. However, inspectors are permitted at any time to increase sampling rates in the event the raw product appears unsound or unsanitary situations arise.

If, after analysis under the normal plan, five (5) consecutive samples contain individually less than 1/4 of the limit for fly eggs and no maggots, the inspector may apply a reduced rate of one (1) count every 6 hours. The normal plan is to be resumed if any sample exceeds this reduced limit.

If at any time a sample exceeds 3/4ths of the limit for fly eggs or contains more than one maggot up to 2mm or any over 2mm, the tightened plan of one count per hour is to be applied for each source of continuous production.

The normal plan may be resumed from the tightened plan after three (3) consecutive samples individually contain no more than 1/2 the guide for fly eggs and no maggots.

Source of Samples

Samples under inplant inspection may be drawn from many sources within an operation. The inspector may at his discretion draw samples at any point in the process where no further quality changes will occur.

* - Changed, July 1968
\+ - Added, July 1968

V PREPARATION OF SAMPLE

A CANNED TOMATOES

1) Normally the size of the aliquot for canned tomatoes is 20 ounces of well mixed juice and the equivalents of a 2-1/2 can of tomato ingredients (loosely packed).

This aliquot is composited as follows, depending upon the individual container size:

CONTAINER SIZE	Number of Containers
8 ounce	Use 6 Containers Per Aliquot
10 ounce	Use 5 Containers Per Aliquot
# 303 and larger	Use 3 Containers Per Aliquot

Can sizes larger than #303: if composite yields less juice or tomato ingredient than specified above use entire amount available.

Can sizes of less then #303 (other than 8 or 10 ounce): use sufficient containers to make 48 to 50 ounces net contents; or a sufficient number to give 20 ounces of juice and a No. 2-1/2 can of tomato ingredient.

Under inplant inspection it is permissible to extract only the juice if the tomatoes have been lye peeled. Inspectors should check the peeled tomatoes in order to assure that no contamination is present and could contribute to higher counts. This can be accomplished by washing representative samples of peeled tomatoes off the canning tables and observing the washing for eggs and maggots. If the topping juice is of a lesser concentration than that of regular tomato juice use a 20 ounce aliquot or the equivalent of a full No. 2 can. In the event the topping juice is tomato juice puree or paste, prepare sample as prescribed for the individual product.

V PREPARATION OF SAMPLE (Continued)

A CANNED TOMATOES (Continued)

2) After the aliquot has been prepared, pour it (tomatoes and juice) onto a U. S. Standard 8 - mesh sieve, placed atop a funnel, which in turn rests in a No. 10 can.

3) Rinse out draining pans and/or empty cans with minimum amount of warm water, adding such rinsings to the No. 10 can, straining through screen on doing so.

4) Shake screen rather forcibly to effectively drain off all liquor and uniformly distribute tomatoes over the screen.

5) Wash tomatoes with a fine but forceful spray (jet) of warm water, using as little water as necessary to accomplish an effective washing.

6) Shake screen again, agitating the tomatoes so that different surfaces are exposed to insure that all portions of the tomatoes are washed.

7) Repeat the washing procedure at least once or as many times as necessary to assure thorough washing of the tomato surfaces.

8) Remove screen, tilt same, and allow any remaining free liquor to drain off into funnel or the No. 10 can.

9) Wash sides of funnel into the No. 10 can, using minimum amount of water.

10) Try to limit drained juice and washings to a volume not in excess of 1/2 the capacity of a No. 10 can. This will permit the use of a single extraction flask.

No change in text

B TOMATO JUICE

Take 100 grams of well-mixed tomato juice from each of three (3) containers of the same code, whenever possible. The entire 300 gram sample thus obtained is the aliquot to use in the examination of tomato juice for fly eggs and maggots.

Proceed as under "Extraction of Sample."

C TOMATO PASTE, or
TOMATO PUREE (TOMATO PULP), or
CONCENTRATED TOMATO JUICE

Take 100 grams of the well-mixed product from each of three (3) containers of the same code, whenever possible. Place in a suitable receptacle of such
* size as to permit addition of enough water to dilute sample to 5.5% natural tomato soluble solids -- Refractive Index 1.3410 @ 20° C. (See dilution chart Inspection Aid 77). From this diluted, thoroughly mixed sample, weigh out a 300-gram sub-sample to use in examining these products for fly eggs.

Proceed as under "Extraction of Sample."

D TOMATO CATSUP

Take 100 grams of the well-mixed catsup from each of three (3) containers of the same code, whenever possible. Add 450 grams of water. (This will comprise a sample of 750 grams net weight.) From this diluted, thoroughly mixed sample, weigh out a 300-gram sub-sample to use in examining catsup for fly eggs.

Proceed as under "Extraction of Sample."

E TOMATO SAUCE

Take 100 grams of the well-mixed tomato sauce from each of three (3) containers of the same code, whenever possible. Add 200 grams of water. (This will comprise a sample of 500 grams net weight.) From this diluted, thoroughly mixed sample, weigh out a 300-gram sub-sample to use in examining tomato sauce for fly eggs.

Proceed as under "Extraction of Sample."

F PIZZA SAUCE

1) Take 100 grams of well-mixed product from each of three (3) containers of the same code and size whenever possible.

2) Dilute sample with 200 grams of water. (This will comprise a sample of 500 grams net weight.)

3) Weigh out 300 grams of the diluted, thoroughly-mixed product.

4) Wash this sample through the 10-mesh sieve into a 2,000 ml. beaker with a fine spray of warm water to remove seeds and peel.

5) Transfer the seeds and peel so removed from the aliquot by the sieve into the black bottom pan and examine macroscopically for maggots. The strained aliquot thus obtained is used in determining number of fly eggs and maggots in this product.

6) Proceed as under "Extraction of Sample."

G CHILI SAUCE

1) Take 100 grams of well-mixed product from each of three (3) containers of the same code and size whenever possible.

2) Dilute sample with 300 grams of water. (This will comprise a sample of 600 grams net weight.)

3) Weigh out 300 grams of the diluted, thoroughly-mixed product.

4) Wash this sample through the 10-mesh sieve into a 2,000 ml. beaker with a fine spray of warm water to remove seeds and peel.

5) Transfer the seeds and peel so removed from the aliquot by the sieve into the black bottom pan and examine macroscopically for maggots.

6) The strained aliquot thus obtained is used in determining number of fly eggs and maggots in this product.

7) Proceed as under "Extraction of Sample."

H SPECIALTY PRODUCTS AND SOUPS

Spaghetti Sauce (all styles)	Spaghetti with Tomato Sauce
Pork and Beans with Tomato Sauce	Tomato Soup
	Cocktail Sauces
Bar-B-Que (and like) Sauces	Tomato Aspic

As a general rule disregard testing of finished product. Under in-process inspection check tomato component prior to formulation and accept or reject. Under lot inspection disregard testing for fly eggs and maggots unless requested to do so, or circumstances would necessitate testing. Contact Washington office for advice on test procedure.

VI EXTRACTION OF SAMPLE

- - - - - - - - - - - - ALL TOMATO PRODUCTS - - - - - - - - - - - - -

A EQUIPMENT

	Kind and Size		Where Obtained 1/
1)	400 ml. beakers	1)	Any reputable chemical apparatus supply house.
2)	2 - liter (pear-shaped) pyrex separatory funnels	2)	Any reputable chemical apparatus supply house.
3)	50 ml. graduate (with 1 ml. graduations)	3)	Any reputable chemical apparatus supply house.
4)	Unleaded gasoline	4)	Any reputable gasoline dealer who sells this particular type of gasoline.
5)	Klerzyme 200	5)	Wallenstein Co. Division of Travenol Laboratories, Inc. Kingstree, South Carolina 29556
	or		
5a)	Pectinol 10-R	5a)	Rohm & Haas Company Independence Mall, West Philadelphia, PA 19105
6)	Suitable suction apparatus --Buchner funnel, suction flask, pressure tubing, etc.	6)	Any reputable chemical apparatus supply house.
7)	Bolting Cloth -- 10XX -- Silk, black	* 7)	Kressilk Products 525 Monterey Pass Road Monterey Park, CA 91754
	or		
7a)	Bolting Cloth -- 10XX -- HC-3-132 Nitex Monofilament Nylon	7a)	H. R. Williams Mill Supply Co. 208 West 19th Street Kansas City, Missouri 64108 Phone (816) 474-1511
8)	S & S No. 10 Ruled Black Filter Paper with blue lines	8)	Schleicher & Schuell Co. Keene, New Hampshire 03431
9)	Stereoscopic microscope	9)	Any available source.

1/ Except where no other material or equipment is suitable, the citation here does not endorse these products to the detriment of others which may be of equal useability.

* Corrected source of supply

B. PREPARATION OF REAGENTS AND MATERIALS

Pectolytic Enzyme

Add six (6) level teaspoonfuls of pectolytic enzyme (any type listed under "Equipment" is equally acceptable) to 100 ml. of warm water, stirring well for two minutes.

Allow to settle until diatomaceous earth settles to bottom, then pour off clear solution.

Make up the solution in multiples of the above proportions.

Prepare a fresh supply daily.

Pectic jellies which may form when making extraction, particularly catsup, may be greatly reduced by including 0.5 gram of potassium oxalate in the above solution.

Bolting Cloth

Black silk (or nylon) bolting cloth 10XX should be used (8XX can be substituted).

Before cutting into the desired shape and size to fit the filtering (suction) apparatus it should be boiled in order to prevent shrinkage.

If not a black color when purchased, the cloth should be dyed a dark blue (or black) to facilitate counting. The boiled, (dyed) cloth may be ruled with red India ink or other permanent marking ink (such as white) with parallel lines 5 to 7 mm. apart.

An alternate aid is to paint a Petri dish with a black enamel and after the paint is dry and hardened, score the surface with a knife or sharp instrument ruling it off in parallel lines 5 to 7 mm. apart. The bolting cloth may then be pressed to the inverted Petri dish and light shining through the scored lines will provide a guide during counting.

Also, counting grids, used in many tomato packing plants for such analyses, are acceptable substitutes.

Black Filter Paper

Ruled black filter paper with blue lines may be used instead of bolting cloth.

This filter paper is preruled, 7 cm. (2-3/4 inches) in diameter and has the same porosity as the bolting cloth.

The filter is used one time and discarded.

No change in text.

Stereoscopic Microscope

The usual binocular stereoscopic scope is satisfactory for counting eggs and maggots. Ordinarily 20 X magnification is sufficient. (Higher magnification may be used for identifying purposes).

As an aid in counting, the scope may be set up with a substage lamp beneath the stage and a spot light directing illumination from above.

If the black Petri dish is used, the substage lamp will provide an excellent source of light that will make the ruled lines very distinct.

C EXTRACTION PROCEDURE

1) Mark code on a two (2) liter separatory funnel.

2) Wash the prepared aliquot into separatory funnel, rinsing container adequately. Add approximately 15 ml. of pectolytic enzyme solution, mix well, and let stand for 15 minutes.

3) Add approximately 30 ml. of white gasoline. Put stopper in place, invert the funnel and release pressure by opening stopcock momentarily.

4) Shake the funnel, held horizontally, with a vigorous back and forth motion for one minute, releasing pressure as necessary.

5) Add warm water to bring the level to the wide portion of the funnel. Mix thoroughly by swirling and inverting funnel several times.

6) Mark time on funnel or use other means to adequately indicate time.

7) Let stand 15 minutes, then drain off into a 400 ml. beaker approximately 20 ml. of the bottom liquid.

8) Repeat swirling, standing for 15 minutes and draining 2 more times. On the last draining, remove approximately 200 ml. of the bottom liquid.

C EXTRACTION PROCEDURE (Continued)

9) Discard the contents of the separatory funnel at this time.

NOTE: If, after the first draining, the trapped-off liquid is filtered and only one fly egg is recovered, the product may be regarded as within the acceptance level for fly eggs and the procedure may be terminated at this point; provided a full "count" of fly eggs is not required or requested. This criteria does not apply to maggots regardless of their size.

10) Filter the trapped-off liquid obtained in Step 7 (under "Extraction of Sample") through the 10XX Bolting Cloth, using suitable suction.

11) Count the fly eggs and maggots on the cloth using a stereoscopic microscope at approximately 20x magnification. Greater magnifications for identification purposes may be used, if necessary.

D RECORDING RESULTS

Record results on any suitable form which will enumerate number of fly eggs and maggots recovered per extraction. Treat each item individually. Recheck code marks to assure that they have been transcribed accurately. For classification purposes and application of guide, it will be necessary to determine and record length of any recovered maggots.

* - Changed, June 1968

VII MAGGOT RECOVERY

The procedure for the macroscopic examination of Canned Tomatoes (and related products) is the basic sedimentation method.

The liquid drained from the Tomatoes (including any seeds and peel which may be retained on the sieve when straining the aliquot for fly egg extraction) is placed in a black bottom pan and the can or container rinsed into the pan using tap water.

Using a generous supply of water, the tomato material is floated off until the bottom of the pan can be readily examined.

The water remaining in the pan is swirled and examined using a strong light.

Maggots over 3mm. in length can be readily seen as they remain on the bottom of the pan and collect near the center * as the water is swirled. However, sometimes when observing for maggots in a black pan, in order to confirm that an object is a maggot, it is necessary to place the object on the bolting cloth to observe under magnification.

The best way to transfer maggots from the black pan to the cloth is by drawing the maggot into an eye dropper and depositing it on the cloth.

Enumerate the number and size of maggots found.

Do not attempt to count fly eggs by this method.

(Ordinarily, we do not expect <u>comminuted</u> tomato products and juice to contain any large (over 3mm.) larvae or maggots since this type of infestation should be removed or disintegrated in the finishers and juice extractors.)

* - Addition, December 1968

VIII LOT ACCEPTANCE

The general policy that each code mark "stand on its own" is applicable to fly egg and maggot contamination as is the same policy on grade and other factors. Therefore, insofar as possible subs for extraction should be made up of a single mark.

Notwithstanding the foregoing there will be times when code marks are so numerous (under lot inspection) that it will not be practical to restrict an extraction to a single mark. In such cases one will not have sufficient information to draw valid conclusions on a single mark and the entire lot must be accepted or failed on the basis of the findings.

A somewhat similar situation exists when the lot consists of multiple codes, each code being extracted separately and one or more marks exceed the guide. The entire lot should be failed and the packer allowed an option to segregate those marks which he thinks are acceptable. However, the "good" portion so segregated must be resampled and retested in order to provide assurance that the segregated portion is acceptable. One must keep in mind that the number of fly eggs recovered from a single code mark can vary considerably from test to test. Only by increasing the number of tests can one be reasonably sure of the estimate of the prevalence of contamination in the mark or the lot.

CONSUMER AND MARKETING SERVICE
FRUIT AND VEGETABLE DIVISION

INSPECTION AID NO. 77
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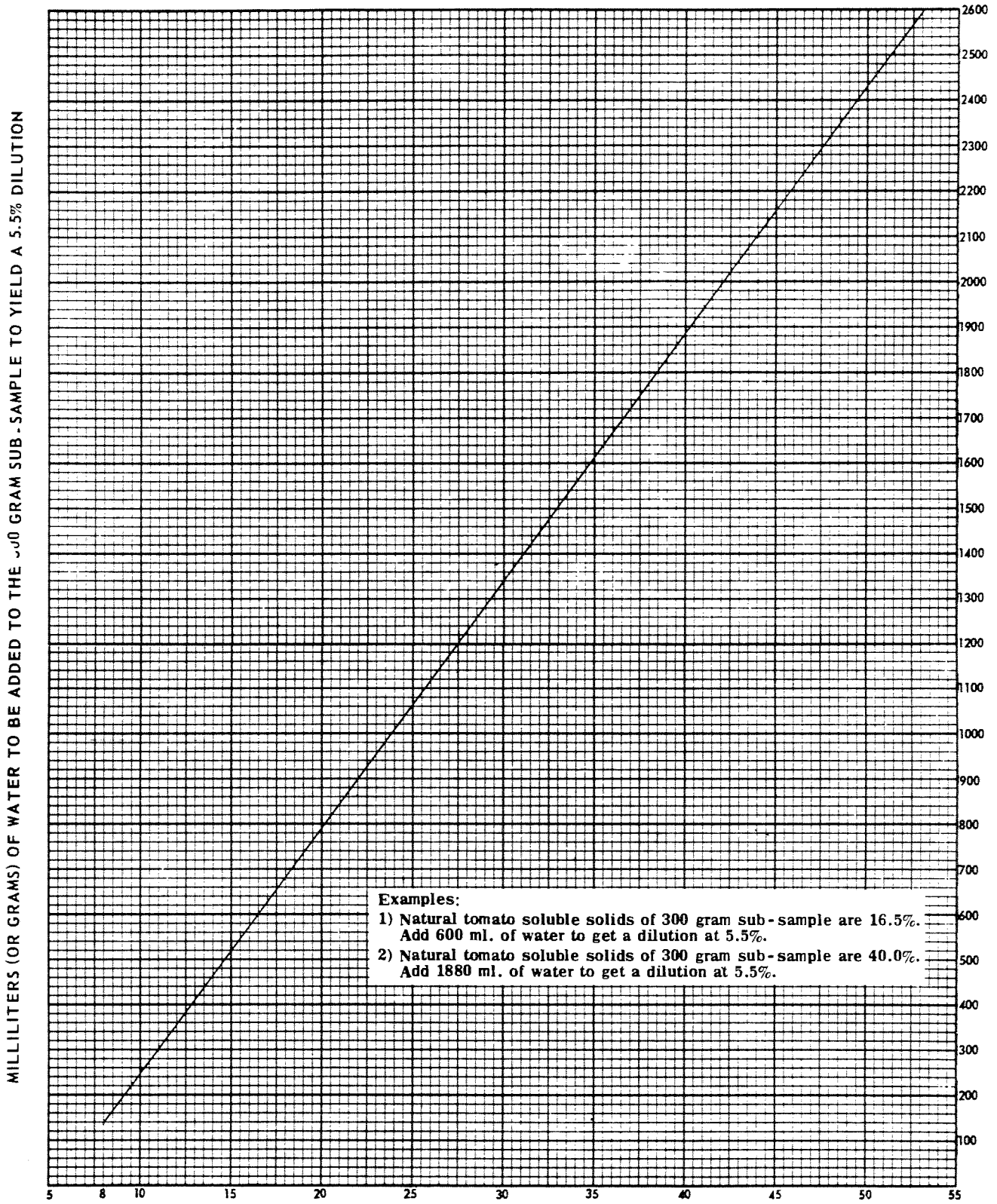
TOMATO PASTE AND TOMATO PUREE

FLY EGG AND MAGGOT DILUTION CHART



Examples:

1) Natural tomato soluble solids of 300 gram sub-sample are 16.5%. Add 600 ml. of water to get a dilution at 5.5%.
2) Natural tomato soluble solids of 300 gram sub-sample are 40.0%. Add 1880 ml. of water to get a dilution at 5.5%.