APPENDIX G. CHEMICAL AND BACTERIOLOGICAL TESTS

I. PRIVATE WATER SUPPLIES AND RECIRCULATED WATER - BACTERIOLOGICAL

Reference: Section 7, Items 8r, 18r, 7p and 17p.
Application: To private water supplies, used by dairy farms, milk plants, receiving stations, transfer stations and milk tank truck cleaning facilities, and to recirculated cooling water, used in milk plants, receiving stations and dairy farms.
Frequency: Initially; after repair, modification or disinfection of the private water supplies of dairy farms, milk plants, receiving stations, transfer stations and milk tank truck cleaning facilities, and thereafter; semiannually for all milk plants, receiving stations, transfer stations and milk tank truck cleaning facilities water supplies and at least every three (3) years on dairy farms. Recirculated cooling water in milk plants, receiving stations and on dairy farms shall be tested semiannually.
Criteria: A MPN of coliform organisms of less than 1.1 per 100 mL, when ten (10) replicate tubes containing 10 mL, or when five (5) replicate tubes containing 20 mL are tested using the Multiple Tube Fermentation (MTF) technique, or one of the Chromogenic Substrate multiple tube procedures; a direct count of less than 1 per 100 mL using the Membrane Filter (MF) technique; or a presence/absence (P/A) determination indicating less than 1 per 100 mL when one vessel containing 100 mL is tested using the MTF technique or one of the Chromogenic Substrate procedures. The Chromogenic Substrate procedures are not acceptable for recirculated cooling water. Any sample producing a bacteriological result of Too Numerous To Count (TNTC) or Confluent Growth (CG) by the MF technique; or turbidity in a presumptive test with no gas production and with no gas production in confirmation (optional test) by the MTF technique (both MPN and P/A format) shall be considered invalid and shall have a Heterotrophic Plate Count (HPC), from the same sample or subsequent resample, of less than 500 colony forming units (CFU) per mL in order to be deemed satisfactory. Findings by HPC shall be reported as Positive or Not-Found.
Apparatus, Methods and Procedure: Tests performed shall conform with the current edition of *SMEWW* or with FDA approved, EPA promulgated methods for the examination of water and waste water or the applicable FDA/NCIMS 2400 Forms. (Refer to M-a-98, latest revision.)
Corrective Action: When the laboratory report on the sample is unsatisfactory, the water supply in question shall again be physically inspected and necessary corrections made until subsequent samples are bacteriologically satisfactory.

II. PASTEURIZATION EFFICIENCY - FIELD PHOSPHATASE TEST

Reference: Section 6.
Frequency: When any laboratory phosphatase test is positive, or any doubt arises as to the adequacy of pasteurization due to noncompliance with equipment, or requirements of Item 16p.
Criteria: Less than 350 mU/L by an electronic phosphatase procedure.
Apparatus: Fluorophos (Advanced Instruments), Paslite and Fast Alkaline Phosphatase (Charm Sciences, Inc.), approved/validated standards and accessories.
Methods: The test is based on the detection of the phosphatase enzyme, a constituent that is inactivated by pasteurization at 63°C (145°F) for thirty (30) minutes or 72°C (161°F) for fifteen

(15) seconds. When pasteurization is faulty, some phosphatase remains and is determined by the electronic detection of fluorescent or chemiluminescent by-products of its action on the approved test system's substrates.

Procedure: Refer to the applicable FDA/NCIMS 2400 Forms and M-a-98, latest revision, for the specific milk and/or milk products for which there are approved phosphatase tests available.

Corrective Action: Whenever a phosphatase test is positive, the cause shall be determined. Where the cause is improper pasteurization, it shall be corrected and any milk or milk products involved shall not be offered for sale.

III. PHOSPHATASE REACTIVATION IN HTST PASTEURIZED PRODUCTS

The presence of an appreciable quantity of phosphatase in milk and cream after heat treatment has been traditionally regarded as evidence of inadequate pasteurization. However, with the advent of modern HTST methods, evidence has been accumulating that under certain conditions, the relationship between inadequate pasteurization and the presence of phosphatase does not hold.

A number of investigators who have studied HTST pasteurizing methods have concluded that while a negative test can be obtained immediately after pasteurization, the same sample may yield a positive test after a short period of storage, particularly if the product is not continuously or adequately refrigerated. This phenomenon has come to be known as reactivation.

Reactivation may occur in HTST pasteurized products, after storage, at temperatures as low as 10°C (50°F), although 34°C (93°F) is optimum. Products of high fat content generally produce relatively more reactivable phosphatase.

Reactivation is greatest in products pasteurized at about 110°C (230°F) but may occur in products pasteurized at much higher temperatures and as low as 73°C (163°F).

It has been noted that an increase in holding time during pasteurization will reduce reactivation.

The addition of magnesium acetate to HTST processed milk or cream, after pasteurization but before storage, accelerates reactivation. The difference in activity between an adequately pasteurized sample, stored with and without magnesium, and an inadequately pasteurized sample, stored with and without magnesium, forms the basis of a test for differentiating reactivated from residual, inadequately pasteurized, phosphatase.

IV. DETECTION OF PESTICIDES IN MILK

Any Regulatory Agency that has adopted this *Ordinance* should operate under a control program that will insure that milk supplies are free from pesticide contamination, in conformance with Section 2.

Pesticide compounds gain access to milk by various routes, including any of the following:

1. Application to the lactating animals;
2. Inhalation of toxic vapors, by the animals, following application to their environment;
3. Ingestion of residues in feed and water; and
4. Accidental contamination of milk, feed and utensils.

At the present time, chlorinated hydrocarbon pesticides are the chief concern. While there are other pest control compounds that are more toxic than the chlorinated hydrocarbons, many of the

agents in this latter group tend to accumulate in the body fat of both lactating animals and human beings, and are secreted in the milk of contaminated lactating animals. The accumulation of these toxic agents in persons continually consuming contaminated milk may reach hazardous concentrations.

Advances in residue analysis have resulted in a radical decrease in the use of paper chromatographic screening procedures for milk, because of its rather limited sensitivity. Regulatory Agencies can now routinely detect residues as low as 0.01 ppm of many of the chlorinated organic pesticides. Satisfactory screening procedures should, therefore, attain this level of sensitivity, which usually necessitates the use of gas chromatography or thin layer chromatography.

General screening procedures of the latter two (2) types are described and discussed in Volume 1 of the *Pesticide Analytical Manual* (*PAM*) published by FDA.

The need for closer scrutiny of milk supplies for pesticide residues has stimulated considerable research in detection technology. The Regulatory Agency entering upon a surveillance program should carefully check the available equipment in relation to its adaptability to the indicated need.

While a schedule of testing comparable to that for microorganisms, four (4) tests of individual producer's milk during any consecutive six (6) months, would be desirable, broad-spectrum procedures are too time consuming to render such a schedule feasible. As a more practical approach, the following procedure is suggested:

1. Test one (1) load of milk from each milk tank truck route, every six (6) months, by a broad spectrum method and trace positive samples; or
2. Test each producer's milk four (4) times every six (6) months for the most common chlorinated hydrocarbon pesticides, by available instrumental methodology.

NOTE: Where Procedure 1 is used, samples of commingled milk from known sources are drawn from receiving station storage tanks. Sampling for Procedure 2 may be done directly from the weigh tank.

V. DETECTION OF DRUG RESIDUES IN MILK

The problem of drug residues in milk is associated with their use in the treatment of mastitis and other diseases. Failure to withhold milk from the market for a sufficient length of time after treatment may result in the presence of drug residues in milk. Such milk is undesirable for two (2) reasons:

1. It comes from an unhealthy lactating animal; and
2. It is adulterated.

The allergenic properties of certain drugs in common use make their presence in milk potentially hazardous to consumers. Also, substantial losses of byproducts may be sustained by the milk industry each year because of the inhibitory effects of drug residues on the culturing process. Drug residues shall be tested for, using tests provided for in Section 6 of this *Ordinance*. These tests are specified in memoranda from the FDA. (Refer to the latest revision of M-a-85 for the approved drug tests, the FDA/NCIMS 2400 Forms for each specific test method and M-a-98,

latest revision, for the specific milk and/or milk products for which there are approved drug tests available.)

VI. ANALYSIS OF MILK AND MILK PRODUCTS FOR VITAMIN A AND D CONTENT

Reference: Section 6.
Frequency: Annually for each product type, or when any doubt arises as to the adequacy of vitamin fortification. (Refer to Appendix O. of this *Ordinance*.)
Methods: Vitamin testing shall be performed using test methods acceptable to FDA and other official methodologies that give statistically equivalent results to the FDA methods. (Refer to M-a-98, latest revision, for the specific milk and/or milk products that have FDA validated and NCIMS accepted test methods for vitamins.)

REFERENCES

Official Methods of Analysis of AOAC INTERNATIONAL, 19th Edition, 2012.

Pesticide Analytical Manual, (*PAM*) available from the U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, HFS-335, 5100 Paint Branch Parkway, College Park, MD 20740-3835.