

FINAL DRAFT
RECOMMENDED CODE OF PRACTICE FOR THE PROCESSING AND
HANDLING OF ETHNIC MILK-BASED CONFECTIONERIES (*PASTILLAS*
AND *YEMA*)

1. SCOPE

This Code of Practice is concerned with the receipt of raw materials and ingredients, preparation and processing of ethnic milk-based confectioneries (*pastillas* and *yema*) as defined in this Code, in order to conform with the required standards stated in PNS/FDA No. ____ Standards for Ethnic Milk-based Confectioneries (*pastillas* and *yema*). The product shall be prepared from a basic mixture of milk and sugar, and eggs (for *yema* only). This Code is intended to provide guidelines to achieve compliance with the standards for ethnic milk-based confectioneries (*pastillas* and *yema*) packed in any suitable container.

2. DEFINITION OF TERMS

For the purpose of this Code, the following definitions apply:

Confectionery - a group of food items primarily made of sugar and other sweeteners, and includes candies, caramels, toffees, and chocolate bars. It is a generic term for sweetened food products. Sugar confectionery refers to products such as sweets, candy and chocolates. These products are shelf-stable and usually have water activity below 0.85. (Dictionary of Food Science and Technology, International Food Information Service, Blackwell Publishing, UK, 2005.)

Container – any form of packaging material, which completely or partially encloses the food (including wrappers). A container may enclose the food as a single item or several units or types of prepackaged food when such is presented for sale to the consumer.

Current Good Manufacturing Practices (cGMP) – a quality assurance system aimed at ensuring that products are consistently manufactured, packed or repacked

or held to a quality appropriate for the intended use. It is thus concerned with both manufacturing and quality control procedures.

Flavor and flavoring substances – substances which are added to impart flavor which are either natural, nature identical or artificial flavoring substances (A.O. No. 88-B s. 1984; Rules and Regulations governing the Labeling of Prepackaged Food Products distributed in the Philippines).

(a) natural flavor

flavoring substances derived through appropriate physical processes from spices, herbs, fruit or fruit juices, vegetable or vegetable juices, edible yeast, bark, bud, root, leaf or plant materials, meat, fish, poultry, eggs, dairy products or fermentation products thereof.

(b) nature-identical flavoring substances

substances chemically derived from aromatic materials or obtained synthetically, which are chemically identical to substances present in' natural products intended for human consumption.

(c) artificial flavoring substances

substances that impart flavor but which have not been identified in natural products or natural sources of flavorings.

Food - any processed substance which is intended for human consumption and includes drink for man, beverages, chewing gum and any substances which have been used as an ingredient in the manufacture, preparation or treatment of food. (RA 9711 Food and Drug Administration (FDA) Act of 2009).

Food Additives – any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food; and including any source of radiation intended for any such use), if such substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures to be safe under the conditions of the intended use (R.A. 3720. Food, Drug and Cosmetic Act).

Food and Drug Administration or FDA - It is formerly known as Bureau of Food and Drug (BFAD) of the Department of Health (DOH); which was renamed in accordance to RA 9711 (Food and Drug Administration (FDA) Act of 2009).

Food Standard – a regulatory guideline that defines the identity of a given food product (i.e. its name and the ingredients used for its preparation) and specifies the minimum quality factors and, when necessary, the required fill of the container. It may also include specific labeling requirements other than or in addition to the labeling requirements generally applicable to all prepackaged foods.

Ingredient - any substance including food additive, used as a component in the manufacture or preparation of a food and present in the final product in its original or modified form.

Label – includes any tag, brand, mark, pictorial, or other descriptive script, written, printed, marked, embossed or impressed on, or attached to the container.

Labeling – any written, printed or graphic matter (1) upon any article or any of its container or wrappers and/or (2) accompanying the packaged food.

Lot – food produced during a period of time and under more or less the same manufacturing condition indicated by a specific code.

Milk – the normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing (CODEX STAN 206-1999).

Milk product – a product obtained by any processing of milk, which may contain food additives, and other ingredients functionally necessary for the processing (CODEX STAN 206-1999).

Packaging – the process of packing that is part of the production cycle applied to a bulk product to obtain the finished product. Any material, including painted material,

employed in the packaging of a product including any outer packaging used for transportation of shipment. Packaging materials are referred to as primary or secondary according to whether or not they are intended to be in direct contact with the product.

Rancidity – formation of off-flavors in food due to lipid oxidation (oxidative rancidity) and/or release of free fatty acids by lipolysis (hydrolytic rancidity).

Water Activity – the ratio of vapor pressure of water in the food substrate to the vapor pressure of pure water at the same temperature (Jay *et. al.*, 2005). It is also a measure of water available for chemical reactions and microbial growth (Fennema, 1996).

3. RAW MATERIALS, INGREDIENTS AND PACKAGING MATERIAL REQUIREMENTS

3.1 Raw materials and ingredients.

Raw materials for processing shall not contain parasites, microorganisms, toxins, and decomposed or extraneous substances.

3.1.1 Basic Ingredient

Milk and milk products. Must conform to requirements prescribed by PNS/BAFPS 36:2008 (Philippine National Standard for Fresh Milk), FDA A.O. No. 132 s. 1970 (Regulation Prescribing the Standard of Identity and Quality of Milk and Milk Products, B-4.12-01), and other applicable food standards.

Milk and milk products used in the production of ethnic milk-based confectioneries (*pastillas* and *yema*) should come from healthy animals. Milk and milk products should have been produced under hygienic conditions as required by the regulatory agency and/or authority for this type of food items.

The preparation of *pastillas* and *yema* may utilize two forms of milk or milk product, namely:

(1) Liquid milk – This may be as fresh, evaporated, condensed milk, and other suitable types of liquid milk.

(2) Powdered milk – This may be as full cream, skimmed powdered milk, and other suitable types of powdered milk.

Sugar and other sweeteners. May include table sugar, corn syrup, and other similar food items, and must conform to all applicable standards.

Eggs. Applicable for *yema* only. Must come from fresh eggs, and must comply with the requirements prescribed by PNS/BAFPS 35: 2005 (Philippine National Standards for Table Egg), and other applicable food standards.

3.1.2 Optional Ingredients

Butter, margarine and other similar food items. Must comply with FDA A.O. No. 243 s. 1975 (Regulation: B-4 Definition and Standards of Food; B-4.18 Margarine), and other applicable food standards.

Fruit, vegetables, nuts, and root crops. May include fresh items or preserves and must conform to all applicable food standards

Water. Only clean, potable water (Annex A) shall be used for the preparation and for all the pretreatment and processing steps of confectionery production.

Non-potable water may be used only for operations not in direct contact with the food materials provided that this does not pose a hazard to health as determined and approved by the official agency having the jurisdiction over it.

Flavor and Flavoring Substances. All flavor/flavoring substances as defined in section 2 shall be certified as food grade by the Food and Drugs Administration (FDA).

Other ingredients. May include starch, cocoa powder, and other ingredients. All other ingredients to be used shall be of food grade quality and conform to all applicable food standards.

3.2 Packaging materials. The primary packaging used shall be made of suitable food-grade and inert materials that would not adversely affect product quality and safety. The packaging materials should be appropriate for the product to be packed and for the expected conditions of handling during distribution and storage. These should provide the products adequate protection from contamination and should be sufficiently durable to withstand mechanical, chemical and thermal stresses encountered during processing and normal distribution. All packaging materials must be clean and free from defects that may affect the product or package integrity. These shall be stored in a clean and sanitary manner.

4. HYGIENE

It is recommended that the product covered by the provisions of this code of practice be prepared and handled in accordance with the appropriate sections of the Recommended International Code of Practice – General Principles of Food Hygiene (CAC/RCP 1–1969, Rev 4 (2003)), Code of Hygienic Practice for Milk and Milk Products (CAC/RCP 57-2004), Recommended International Code of Hygienic Practice for Egg Products CAC/RCP 15-1976 (amended 1978, 1985), and/or the BFAD A.O. No. 153 s. 2004 - Guidelines, Current Good Manufacturing Practices in Manufacturing, Packing, Repacking or Holding Food, covering the plant facilities and operations requirement including the construction and layout of processing plant, hygienic facilities, equipment, utensils and working surfaces.

5. PREPARATION AND PROCESSING

The preparation of ethnic milk-based confectioneries (*pastillas* and *yema*) is described from the receipt of raw materials until the packing operations. The production process should be supervised by personnel with adequate technical training and experience.

5.1 Preparation of Raw Materials and Ingredients

5.1.1 Milk and Milk Products

Receipt

Milk and milk products shall only be accepted if it is sound and suitable for processing, according to the requirements stipulated in sub-subsection 3.1.1. Those found with contamination should be rejected. Special precautions must be taken to reject milk and milk products showing signs of spoilage, and deterioration.

If prepackaged processed milk products are to be used as raw material, choose only those contained in clean, non-toxic, and properly labeled packaging materials.

Storage/holding

Milk and milk products held for processing should be stored in any suitable type of closed container to protect from domestic animals, parasites, chemical or microbiological contaminants, debris, and dust.

The storage temperature for milk and milk products should be sufficient to maintain product safety and suitability. Fresh milk and similar fluid milk products must be stored at temperatures less than 7°C. Canned and dried milk products may be stored at a cool, dry place.

5.1.2 Sugar and other sweeteners

Receipt and Inspection

Sugar and sweeteners used for the production of ethnic milk-based confectioneries (*pastillas* and *yema*) shall only be accepted if it is sound and suitable for processing, according to the requirements stipulated in sub-subsection 3.1.1. Those found with contamination which could not be removed to acceptable levels by normal plant sorting or preparation procedures should be rejected.

Sugars and other sweeteners must conform to applicable, existing standard specifications for white sugar (PNS/BAFPS 82:2010), raw cane sugar (PNS/BAFPS 81:2010), coconut sap sugar (PNS/BAFPS 76:2010), and other types of sweeteners. Choose only those contained in clean, non-toxic, and properly labeled packaging materials.

Storage/holding

Sugars and sweeteners held for processing should be stored in any suitable type of closed container to protect from domestic animals, parasites, chemical or microbiological contaminants, debris, and dust. These ingredients should be stored at temperature and relative humidity conditions that will minimize deterioration of quality. The storage area should also be free from strong odors or aroma. Regular inspection of the storage facility should be done to avoid infestation.

5.1.3 Eggs

Receipt

Egg used for the production of ethnic milk-based confectioneries (*pastillas* and *yema*) shall only be accepted if it is fresh and suitable for processing, according to the requirements stipulated in sub-subsection 3.1.1. Those found with contamination which could not be removed to acceptable levels by normal plant sorting or preparation procedures should be rejected. Special precautions must be taken to reject eggs with broken shells, or those with signs of degradation, abnormal color or odor.

Inspection and sorting

Eggs shall be inspected and sorted according to quality and size specifications before processing (PNS/BAFPS 35:2005. Philippine National Standard: Table Egg). Choose only those contained in clean, non-toxic, and properly labeled packaging materials.

Storage/holding

Eggs held for processing should be stored in any suitable type of closed container to protect from domestic animals, parasites, chemical or microbiological contaminants, debris, and dust.

Eggs should be stored at temperature and relative humidity conditions that will minimize deterioration of egg quality. Eggs are kept at refrigerated temperatures during storage particularly when these are not immediately used for processing. The

storage area should also be free from strong odors or aroma. Regular inspection of the storage facility should be done to avoid infestation.

Washing and/or sanitizing

Eggs are washed to remove dirt, dust, soil, insect, and filth that might contaminate or affect the color, aroma or flavor of the eggs. Water used for washing and rinsing should be of potable quality. Only approved sanitizing agents and permissible limits may be used in the wash or rinse water for eggs.

5.1.4 Optional Ingredients

Receipt

Optional ingredients to be used in the preparation of ethnic milk-based confectioneries (*pastillas* and *yema*) shall conform to the requirements stipulated in sub-subsection 3.3.2. Whenever applicable, certificates of analyses (COA) from ingredient suppliers shall be secured to confirm their suitability for processing. Ingredients shall be rejected if they do not conform to the requirements and are found to have signs of deterioration, decomposition, or contamination to an extent which renders them unfit for human consumption.

Storage/Holding

Optional ingredients shall be in closed containers as protection against infestation by domestic animals, parasites, filth, and chemical and microbiological contaminants. Storage requirements such as temperature and humidity may vary depending on the ingredient, and these should be provided accordingly by the storage facilities to be used.

Stored stocks of ingredients should be used on a “first in-first out” (FIFO) or a “first to expire-first to use” (FEFU) basis.

5.2 Processing Operations

5.2.1 Preparation

If raw milk is used as basic ingredient, pasteurization must be done to ensure the safety of the product. Sufficient time and temperature combinations for pasteurization must be used to ensure that the milk used does not pose a health hazard. Pasteurization may be done by heating the milk at 63–66°C for at least 30–32 minutes.

Under FDA A.O. No. 132 s. 1970 (Regulation Prescribing the Standard of Identity and Quality of Milk and Milk Products, B-4.12-01), pasteurized milk should have been subjected to a temperature not lower than 63°C and held continuously at that temperature for not less than 30 minutes. Pasteurization could also be done by keeping the milk temperature at not lower than 72°C for at least 15 seconds.

Breaking eggs, and/or separating the egg yolk from the egg white must be performed in a hygienic manner to prevent contamination. Special precaution must be taken to reject eggs with abnormal odor, color, or appearance.

5.2.2 Mixing and/or Cooking

Mix the ingredients together in a saucepan or a double boiler. Cook over low to moderate heat. Do not allow mixture to boil. Constantly stir the mixture to avoid burning or scorching the mixture. Stir and cook the product mixture until a thick paste is formed. At this point, the consistency of the mixture should have thickened enough that it separates from the sides of the pan. Allow the mixture to cool.

For *pastillas*, some formulations that utilize milk products (e.g. condensed or powdered milk) do not require cooking. Instead, the ingredients are simply mixed together and then kneaded manually until firm enough to handle.

5.2.3 Forming and Wrapping

Divide the mixture into equal portions. For *pastillas*, each portion could be formed into sticks or cylinders, or shaped into balls. The formed mixture may be rolled in sugar. The formed *pastillas* may then be individually wrapped in suitable primary packaging such as uncolored cellophane, wax paper, and aluminum foil.

For *yema*, a portion of the cooked mixture may be placed in a piece of cellophane paper, and formed into pyramid-like shapes. The cooked mixture could also be shaped into balls, and either rolled in sugar or dipped in caramel glaze. The *yema* balls may then be individually wrapped in suitable primary packaging such as uncolored cellophane, wax paper, and aluminum foil.

5.3 Packing

Packing can be done either mechanically or manually. It is important to standardize filling for economic reasons. Gas-packing or vacuum-packing may be done.

5.4 Closing or Sealing of Containers

Seams and other closures shall be sealed air-tight to meet the requirements of the processors.

The seal area of flexible containers must be free of food material and wrinkles. Sealing temperature and pressure shall conform to the sealing equipment to be used.

5.5 Coding of Sealed Containers

Coding of sealed container shall be indelible with details of production date and time, batch code, product code, the product line in which product is packed, the manufacturing plant and other information necessary for product traceability. Where the container does not permit the code to be embossed or inked, the label shall be legibly perforated or otherwise marked, and securely affixed to the product container.

5.6 Post-Process Container Handling

Mechanical shocks leading to breakage of semi-rigid containers due to container abuse must be avoided. These occur by knocking against each other during conveying, packaging and labeling operations, among others.

Flexible containers/pouches shall be handled singly rather than in bunches, and care must be exercised so as to prevent damage by roughened contact surfaces.

6. FOOD ADDITIVES

6.1 Food additives when used shall be in accordance with the regulations established by the Food and Drugs Administration (FDA) (Bureau Circular No. 016 s.2006. Updated List of Food Additives) and/or the Codex Alimentarius Commission.

The following food additives listed in, but not limited to, Table 1, may be used for the manufacture of ethnic milk-based confectioneries (*pastillas* and *yema*).

6.2 All others that have not been included in the above list shall be allowed as carry-over provided they are approved by FDA regulation (B.C. No. 016 s. 2006; Updated List of Food Additives) and shall be in accordance to the Section 4 of the Preamble of the General Standard for Food Additives (GFSA) (Codex Stan 192-1995, Rev. 5 (2004)). These additives include those that are used for the raw materials and other ingredients.

Table 1. Food Additives for Ethnic Milk-based Confectioneries (*Pastillas* and *Yema*)* (B.C. No.016 s. 2006. Updated List of Food Additives)

Function	Food Additive	Max. level of usage
Anti-caking agent	Polydimethylsiloxane	10 mg/kg**
		50 mg/kg***
Antioxidant	BHA	100 mg/kg (Fat or oil basis)**
		200 mg/kg (Fat or oil basis)**
		2 mg/kg ***
	BHT	200 mg/kg (Fat or oil basis)**
		90 mg/kg (On dry ingredient, dry weight, dry mix or concentrate basis) ***
	Gallate, propyl	200 mg/kg (Fat or oil basis) **
		90 mg/kg (On dry ingredient, dry weight, dry mix or concentrate basis) ***
	Tertiary Butylhydroquinone	200 mg/kg (Fat or oil basis)**
	Tocopherols	500 mg/kg (Fat or oil basis)**
		150 mg/kg***
Color	Allura Red AC	348 mg/kg**
		300 mg/kg ***
	Amaranth	100 mg/kg **
		300 mg/kg ***
	Annatto Extracts	25 mg/kg (As total bixin or norbixin) **
		10 mg/kg ***
	Brilliant Blue FCF	300 mg/kg**
		150 mg/kg***
	Caramel Colour, Class III	GMP**
		GMP***
	Caramel Colour, Class IV	GMP**
		GMP***
	Fast Green FCF	100 mg/kg**
		100 mg/kg***

	Sunset Yellow FCF	400 mg/kg** 300 mg/kg***
Preservative	Benzoates	1500 mg/kg (As benzoic acid) ** 1000 mg/kg (As benzoic acid)***
		Hydroxybenzoates, p-
Stabilizer	Polysorbates	10000 mg/kg** 5000 mg/kg***
		Sorbates
	Sorbitan Esters of Fatty Acids	
		Sweetener
Acesulfame Potassium	3500 mg/kg ** 350 mg/kg***	
	Aspartame	
Saccharin		
	Sucralose	

*Based on the Food Category System: 5.0 Confectionery;

** Based on the Food Category System:5.2 Sugar-based confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3, and 05.4;

*** Based on the Food Category System:10.4 Egg-based desserts (e.g. custards)

7. LABELLING

7.1 Each container shall be labeled and marked with the following information in accordance with FDA's Labeling Regulation (A.O. 88-B s. 1984; Rules and Regulations

governing the Labeling of Prepackaged Food Products Distributed in the Philippines):

(a) The name of the product shall be "*Pastillas*" (Milk Candy) or "*Yema*" (Custard Candy). Additional descriptors pertaining to the ingredients used or the product form may also be included (e.g. "*Pastillas de Leche*", "*Ube Pastillas*", "*Yema balls*"). Other local or regional names referring to products similar to those defined in 3.1 may also be included, provided that these names are acceptable in the area of distribution.

(b) The complete list of ingredients and food additives used in the preparation of the product in descending order of proportion.

(c) The net quantity of content by weight in the metric system. Other systems of measurement required by importing countries shall appear in parenthesis after the metric system unit.

(d) The name and address of the manufacturer, packer and/or distributor of the food.

(e) Open date marking

The words “Consume Before” or “Expiry Date” indicating end of period at which the product shall retain its optimum quality attributes at defined storage conditions.

(f) Lot or code number identifying product lot.

(g) The words “Product of the Philippines”, or the country of origin if imported.

(h) Additional requirements

A pictorial representation of raw material or end-product on the label should not mislead the consumer with respect to the raw material or end-product so illustrated.

(i) Optional information

Storage instructions may also be indicated on the label.

7.2 Nutrition Labeling

Nutrition labeling shall conform to the established regulations of FDA and/or authority.

8. QUALITY ASSURANCE

8.1 Inspection of Finished Products

All processed products shall be inspected before labeling and casing and defective products shall be withdrawn or rejected. The company must have an approved policy and procedures based on the BFAD A.O. No. 153 s. 2004 – Guidelines on the Current Good Manufacturing Practices in Manufacturing, Packing, Repacking or Holding Food.

8.2 Record Keeping

Permanent and legible dated records of time, temperature code mark and other pertinent

details shall be kept concerning each load. Such records are essential as a check on processing operations.

Written records of all container closure examinations shall specify the code lot, the date and time of container closure inspections, the measurements obtained and all the corrective actions taken.

Records shall be maintained identifying initial distribution of the finished product to facilitate, if necessary, the segregation of specific food lots that may have been contaminated or otherwise unfit for intended use.

All process deviations involving failure to satisfy the minimum requirements of the process shall be recorded detailing those deviations and the actions taken.

8.3 Good Manufacturing Practices (GMP)

Processing establishments shall have developed, documented and implemented prerequisite programs based on BFAD's Current Good Manufacturing Practices (cGMP) and Hygiene Control. An effective GMP and Hygiene Control program will decrease the number of critical control points that a manufacturer must face during the hazard analysis of the product/process.

9. STORAGE AND TRANSPORT OF FINISHED PRODUCT

Storage and transport conditions of the finished product shall be such that the integrity of the product container, and the safety and quality of the product are not adversely affected.

Cases and cartons must be thoroughly dry. They must be of proper size so that the containers fit snugly and are not subject to damage from movement within the case. They must be strong enough to withstand normal transport.

Extreme fluctuations in temperature and humidity during storage and transport of the product must be avoided to prevent product deterioration.

10. LABORATORY CONTROL PROCEDURES

Each food processing establishment shall have access to laboratory control of both the processes used and the finished products. All food ingredients and food products declared unfit for human consumption by the laboratory shall be rejected.

Representative samples for each lot or batch shall be taken to assess the safety and quality of the product.

Microbiological laboratory shall be separated from the processing area. No pathogens shall be handled within the premises of manufacturing plant.

Laboratory procedures for quality control of the processes and the product must follow recognized or standard methods for easy interpretation of results.

11. END PRODUCT SPECIFICATIONS

Appropriate methods shall be used for sampling analysis and determinations to meet the following specifications:

1. To the extent possible in good manufacturing practices, the products shall be free from any objectionable characteristics.
2. The product shall not contain any toxic substances originating from microorganisms and chemicals.
3. The product shall be free from chemical pollutants in amounts which may pose hazard to health.
4. The product shall comply with the requirements set forth by the Food and Drugs Administration, and the Codex Alimentarius Commission on Veterinary Drug Residues and Food Additives.

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ANNEX A
Standard Parameters and Values for Drinking Water
 Philippine National Standards for Drinking Water 2007 (DOH AO 2007-0012)

Table 1. Standard values for bacteriological quality

Parameter	Value/Unit	Point of Compliance
Total Coliform	< 1.1 MPN/100 ml	Service Reservoir Water treatment works Consumers' taps Refilling stations Water haulers Water vending machines
Fecal Coliform	< 1.1 MPN/100 ml	Service Reservoir Water treatment works Consumers' taps Refilling stations Water haulers Water vending machines Point sources - Level 1
Heterotrophic Plate Count	< 500 CFU/ml	Service Reservoir Water treatment works Consumers' taps nearest meter Refilling stations Water vending machines

Table 2. Standard values for Physical and Chemical Quality for Acceptability Aspects for Drinking Water

Constituents	Maximum Level (mg/L) or Characteristic	Constituents	Maximum Level (mg/L) or Characteristic
Taste	No objectionable taste	Hydrogen Sulfide	0.05
Odor	No objectionable odor	Iron	1.0
Color	Apparent = 10 color units True = 5 color units	Manganese	0.4
Turbidity	3 NTU	pH	6.5 – 8.5
Aluminum	0.2	Sodium	200
Chloride	250	Sulfate	250
Copper	1.0	Total Dissolved Solids	500
Hardness	300 as CaCO ₃	Zinc	5.0

Table 3. Standard Values for Organic and Inorganic Chemical Constituents of Health Significance in Drinking Water

Inorganic Chemicals

Constituents	Maximum Level (mg/L)	Constituents	Maximum Level (mg/L)
Antimony	0.02	Fluoride	1.0
Arsenic	0.05	Lead	1.01
Barium	0.7	Mercury (total)	0.001
Boron	0.5	Nickel	0.02
Cadmium	0.003	Nitrate	50
Chromium (Total)	0.05	Nitrite	3.0
Cyanide (Total)	0.07	Selenium	0.01

Organic Chemicals

Constituents	Maximum Level (mg/L)	Constituents	Maximum Level (mg/L)
Benzene	0.01	Ethylbenzene	0.30
Carbon tetrachloride	0.004	Nitrilotriacetic acid (NTA)	0.20
1,2-Dichlorobenzene	0.1	Polyaromatic hydrocarbons (PAHs)	0.20
1,4-Dichlorobenzene	0.5	Polynuclear aromatic	0.0007
1,2-Dichloroethane	0.003	Tetrachloroethene	0.02
1,1-Dichloroethene	0.05	Styrene	0.04
1,2-Dichloroethene	0.07	Tetrachloroethene	0.70
Dichloromethane	1.0	Trichloroethene	0.07
Di(2-ethylhexyl) phthalate	1.01	Vinyl chloride	0.0003
Edetic Acid (ADTA)	0.001	Xylene	0.5

Organic Pesticides

Constituents	Maximum Level (ug/L)	Status in the Philippines
Aldrin and Dieldrin (combined)	30.0	Banned
Atrazine	0.03	Registered
Carbofuran	2.0	Registered
Chlordane	7.0	Banned
DDT **	0.2	Banned
1,2-Dibromo-3-chloropropane (DBCP)	1.0	Banned
2,4-Dichlorophenoxyacetic acid (2,4-D)	1.0	Registered
Endrin	30.	Banned
1,2-Dibromomethane (Ethylene dibromide)	0.6	Banned
Heptachlor and Heptachlor epoxide (combined)	0.03	Banned
Lindane	2.0	Restricted
MCPA (4-(2-methyl-4-chloro) phenoxy) acetic acid	2.0	Registered
Pendimethalin	20.0	Registered
Pentachlorophenol (PCP)	9.0	Banned

ANNEX B

Determination of Water Activity (AOAC 978.18)

A. Principle

Water activity, a_w , is ratio of vapor pressure of H₂O in product to vapor pressure of pure H₂O at same temperature. It is numerically equal to 1/100 of relative humidity (RH) generated by product in closed system. RH can be calculated from direct measurement of partial vapor pressure or dew point or measured indirectly by sensors whose physical or electric characteristics are altered by RH to which they are exposed. Instruments are checked or calibrated on basis of RH generated by standard salt slushes.

B. Instruments and Systems

(Select 1 of following instruments or systems to perform test. Each has different application limitations because of interferences from other volatile components of products being measured. check with instrument manufacturer for more specific limitations.)

- (a) Change in electrical conductivity of immobilized salt solution. – Instrument available from Beckman Industrial, Rosemount Analytical Div., 89 Commerce Rd, Cedar Grove, NJ 07009; Nova Sina AG, Andreastrasse 7-11, CH 8050, Zurich, Switzerland; Rotronic Instrument Corp., 160 E. Main St, Huntington, NY 11743. Immobilized salt sensors are affected by polyols such as glycerol and glycol and by volatile amines
- (b) Change in electrical capacitance of polymer thin films. – Instrument available from General Eastern Instruments, 50 Hunt St, Watertown, MA 02172. Polymer thin film sensors are affected by CH₃COOH.
- (c) Dew point by chilled mirror technique. – Instrument available from EG&G, Environmental Equipment Division, 217 Middlesex Turnpike, Burlington, MA 01803 or General Eastern Instruments. Dew point measurements can be affected by condensables with lower critical temperature than H₂O.
- (d) Longitudinal change in dimensions of water-sorbing fiber. – Instrument available from G Lufft Metallbarometerfabrik, D-7, Postfach 692, Neue Weinsteige 22, Stuttgart, Germany.
- (e) Partial water vapor pressure by manometric system. – Partial H₂O vapor pressure measurements can be made useless by living products that respire, such as grains or nuts; by active fermentation; or by products that expand excessively when subjected to high vacuum.
- (f) Relative weight of moisture sorbed by anhydrous hydrophilic solid, e.g., microcrystalline cellulose.-see J. Agr. Food chem. 22, 326(1974).

C. Apparatus and Reagents

(As needed for instrument or system selected.)

- (a) Dew point instrument. – Equipped to measure temperature to $\pm 0.1^\circ$. See 978.18B(c).
- (b) Forced-draft cabinet. – Constant temperature, set to maintain $25 \pm 1^\circ$; capacity $\geq 0.06 \text{ m}^3$ (2 cu ft); with access port to accommodate instrument sensor leads. Use in conjunction with (c).
- (c) Insulated box with cover. – Large enough to hold test container, (e), and small enough to fit in forced-draft cabinet, (b); with access port to accommodate instrument sensor leads. Protect test container from short-term temperature fluctuations.
- (d) Manometric system. – Sensitive to pressure differential of $\pm 0.01 \text{ mm Hg}$ (1.33 Pa). See 978.18B(e).
- (e) Test containers. – 120 or 240 mL (4 or 8 oz) wide-mouth or Mason glass jars with Al- or Teflon-lined screw caps and gaskets. Check integrity of cap seals and sensor leads by any means available, e.g., ability of system to hold vacuum, using Tesla coil.
- (f) Water bath. – Capable of maintaining temperature constant within 0.1° at $25 \pm 1^\circ$; capacity sufficient to hold measuring chamber of selected apparatus.

(g) Hydrophilic solid. – Microcrystalline cellulose, Type PH-101 (FMC Corp., Pharmaceutical and Bioscience Division, 1735 Market St, Philadelphia, PA 19103, or equivalent).

(h) Reference salts. – ACS reagent grade, fine crystal. see Table 978.18.

Table 978.18 Water Activity of Reference Salt Slushes at 25°

Salt	a_w	Salt	a_w
MgCl ₂	0.328	KBr	0.809
K ₂ CO ₃	0.432	(NH ₄) ₂ SO ₄	0.810
Mg(NO ₃) ₂	0.529	KCl	0.843
NaBr	0.576	Sr(NO ₃) ₂	0.851
CoCl ₂	0.649	BaCl ₂	0.902
SrCl ₂	0.709	KNO ₃	0.936
NaNO ₃	0.743	K ₂ SO ₄	0.973
NaCl	0.753		

D. Preparation of Reference Salt Slushes

Place selected reference salt in test container to depth of ca 4 cm for more soluble salts (lower a_w), to depth of ca 1.5 cm for less soluble salts (higher a_w), and to intermediate depth for intermediate salts. Add H₂O in ca 2 mL increments, stirring well with spatula after each addition, until salt can absorb no more H₂O as evidenced by free liquid. Keep free liquid to minimum needed to establish saturation of salt with H₂O. Slushes are ready for use upon completion of mixing, and are usable indefinitely (except for some high a_w salts susceptible to bacterial attack), if contained in manner to prevent substantial evaporation losses. Some slushes, e.g., NaBr, may solidify gradually by crystal coalescence, with no effect on a_w .

E. Calibration

Select ≥ 5 salts to cover a_w range of interest or range of sensor being used. Measure humidity generated by each salt slush in terms of instrument readout, as in 978.18F. Plot readout against a_w values given in Table 978.18 for selected salts, using cross-section paper scaled for reading to 0.001 a_w unit. Draw best average smooth line through plotted points. Use this calibration line to translate sensor instrument readout of samples to a_w or to check vapor pressure or dew point instruments for proper functioning.

F. Determination

Place calibration slush or sample in forced-draft cabinet, (b), or H₂O bath, (f), until temperature is stabilized at 25 \pm 1°. Transfer salt slush or sample to test container, (e), seal container with sensing device attached, and place in temperature control device. Use volume of sample or slush $>1/20$ total volume sample container plus any associated void volume of sensing system, but not so much as to interfere with operation of system. Record instrument response at 15, 30, 60, and 120 min after test container is placed in temperature control device, or record response on strip chart. Two consecutive readings, at indicated intervals, which vary by <0.01 a_w unit are evidence of adequately close approach to equilibrium. Continue readings at 60-min intervals, if necessary. Convert last reading to a_w by calculation from physical measurements or by reference to calibration line. Make all measurements within range of calibration points; do not extrapolate calibration line. Make all measurements in same direction of change, and, if required by properties of sensor, expose sensor to controlled RH below ambient before starting each measurement.

ANNEX C

Enumeration of Yeast and Mold Count

Enumeration of Yeasts and Molds in Food--Dilution Plating Technique (USFDA, 2001)

A. Equipment and materials

1. Basic equipment (and appropriate techniques) for preparation of sample homogenate
2. Equipment for plating samples
3. Incubator, 25°C
4. Arnold steam chest
5. pH meter
6. Water bath, 45 ± 1° C

B. Media and reagents

1. Dichloran rose bengal chloramphenicol (DRBC) agar
2. Dichloran 18% glycerol (DG18) agar
3. Plate count agar (PCA), standard methods; add 100 mg chloramphenicol/liter when this medium is used for yeast and mold enumeration. This medium is not efficient when "spreader" molds are present.
4. Malt agar (MA)
5. Malt extract agar (Yeasts and Molds) (MEAYM)
6. Potato dextrose agar (PDA), dehydrated; commercially available

C. Procedures

Sample preparation

Analyze 25-50 g from each subsample; generally, larger sample sizes increase reproducibility and lower variance compared with small samples. Test individual subsamples or composite according to respective Compliance Program for the food under analysis. Add appropriate amount of 0.1% peptone water to the weighed sample to achieve 10⁻¹ dilution, then homogenize in a stomacher for 2 min. Alternatively, blending for 30-60 sec can be used but is less effective. Make appropriate 1:10 (1+9) dilutions in 0.1% peptone water. Dilutions of 10⁻⁶ should suffice.

Plating and incubation of sample

Spread-plate method. Aseptically pipet 0.1 ml of each dilution on pre-poured, solidified DRBC agar plates and spread inoculum with a sterile, bent glass rod. DG18 is preferred when the water activity of the analyzed sample is less than 0.95. Plate each dilution in triplicate.

Pour-plate method. Use sterile cotton-plugged pipet to place 1.0 ml portions of sample dilution into prelabeled 15 x 100 mm Petri plates (plastic or glass), and immediately add 20-25 ml tempered DG18 agar. Mix contents by gently swirling plates clockwise, then counterclockwise, taking care to avoid spillage on dish lid. After adding sample dilution, add agar within 1-2 min; otherwise, dilution may begin to adhere to dish bottom (especially if sample is high in starch content and dishes are plastic) and may not mix uniformly. Plate each dilution in triplicate.

From preparation of first sample dilution to pouring or surface-plating of final plate, no more than 20 min (preferably 10 min) should elapse. **Note:** Spread plating of diluted sample is considered better than the pour plate method. When the pour plate technique is used, fungal colonies on the surface grow faster and often obscure those underneath the surface, resulting in less accurate enumeration. Surface plating gives a more uniform

growth and makes colony isolation easier. DRBC agar should be used for spread plates only.

Incubate plates in the dark at 25°C. Do not stack plates higher than 3 and do not invert. **Note:** Let plates remain undisturbed until counting.

Counting of plates

Count plates after 5 days of incubation. If there is no growth at 5 days, re-incubate for another 48 h. Do not count colonies before the end of the incubation period because handling of plates could result in secondary growth from dislodged spores, making final counts invalid. Count plates containing 10-150 colonies. If mainly yeasts are present, plates with 150 colonies are usually countable. However, if substantial amounts of mold are present, depending on the type of mold, the upper countable limit may have to be lowered at the discretion of the analyst. Report results in colony forming units (CFU)/g or CFU/ml based on average count of triplicate set. Round off counts to two significant figures. If third digit is 6 or above, round off to digit above (e.g., 456 = 460); if 4 or below, round off to digit below (e.g., 454 = 450). If third digit is 5, round off to digit below if first 2 digits are an even number (e.g., 445 = 440); round off to digit above if first 2 digits are an odd number (e.g., 455 = 460). When plates from all dilutions have no colonies, report mold and yeast counts (MYC) as less than 1 times the lowest dilution used.

Isolate individual colonies on PDA or MA, if further analysis and species identification is necessary.

ANNEX D

Isolation of *Salmonella* (USFDA, 2001)

A. Sample Preparation

For candy and candy coating (including chocolate). Aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile, reconstituted nonfat dry milk and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2 . Add 0.45 ml 1% aqueous brilliant green dye solution and mix well. Loosen jar caps 1/4 turn and incubate 24 ± 2 h at 35°C . Continue as in B., below.

For egg-containing products (noodles, egg rolls, macaroni, spaghetti), cheese, dough, prepared salads (ham, egg, chicken, tuna, turkey), fresh, frozen, or dried fruits and vegetables, nut meats, crustaceans (shrimp, crab, crayfish, langostinos, lobster), and fish. Preferably, do not thaw frozen samples before analysis. If frozen sample must be tempered to obtain analytical portion, thaw below 45°C for <15 min with continuous agitation in thermostatically controlled water bath or thaw within 18 h at $2-5^\circ\text{C}$.

Aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile lactose broth and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2 . Mix well and loosen jar cap about 1/4 turn. Incubate 24 ± 2 h at 35°C . Continue as in B., below.

B. Isolation of *Salmonella*

1. Tighten lid and gently shake incubated sample.

Guar gum and foods suspected to be contaminated with *S. Typhi*. Transfer 1 ml mixture to 10 ml selenite cystine (SC) broth and another 1 ml mixture to 10 ml TT broth. Vortex.

All other foods. Transfer **0.1** ml mixture to 10 ml Rappaport-Vassiliadis (RV) medium and another 1 ml mixture to 10 ml tetrathionate (TT) broth. Vortex.

2. Incubate selective enrichment media as follows:

Foods with a high microbial load. Incubate RV medium 24 ± 2 h at $42 \pm 0.2^\circ\text{C}$ (circulating, thermostatically-controlled, water bath). Incubate TT broth 24 ± 2 h at $43 \pm 0.2^\circ\text{C}$ (circulating, thermostatically-controlled, water bath).

Foods with a low microbial load (except guar gum and foods suspected to be contaminated with *S. Typhi*). Incubate RV medium 24 ± 2 h at $42 \pm 0.2^\circ\text{C}$ (circulating, thermostatically controlled, water bath). Incubate TT broth 24 ± 2 h at $35 \pm 2.0^\circ\text{C}$.

Guar gum and foods suspected to be contaminated with *S. Typhi*. Incubate SC and TT broths 24 ± 2 h at 35°C .

3. Mix (vortex, if tube) and streak 3 mm loopful (10 μl) incubated TT broth on bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, and Hektoen enteric (HE) agar.

Prepare BS plates the day before streaking and store in dark at room temperature until streaked.

4. Repeat with 3 mm loopful (10 μ l) of RV medium (for samples of high and low microbial load foods) and of SC broth (for guar gum).
5. Refer to 994.04 in *Official Methods of Analysis* (1) for option of refrigerating incubated sample preenrichments and incubated sample selective enrichments (SC and TT broths only) of low moisture foods. This option allows sample analyses to be initiated as late as Thursday while still avoiding weekend work.
6. Incubate plates 24 ± 2 h at 35°C .
7. Examine plates for presence of colonies that may be *Salmonella*.
8. Lightly touch the very center of the colony to be picked with sterile inoculating needle and inoculate TSI slant by streaking slant and stabbing butt. Without flaming, inoculate LIA slant by stabbing butt twice and then streaking slant. Since lysine decarboxylation reaction is strictly anaerobic, the LIA slants must have deep butt (4 cm). Store picked selective agar plates at $5-8^{\circ}\text{C}$.
9. Incubate TSI and LIA slants at 35°C for 24 ± 2 h. Cap tubes loosely to maintain aerobic conditions while incubating slants to prevent excessive H_2S production. *Salmonella* in culture typically produces alkaline (red) slant and acid (yellow) butt, with or without production of H_2S (blackening of agar) in TSI. In LIA, *Salmonella* typically produces alkaline (purple) reaction in butt of tube. Consider only distinct yellow in butt of tube as acidic (negative) reaction. Do not eliminate cultures that produce discoloration in butt of tube solely on this basis. Most *Salmonella* cultures produce H_2S in LIA. Some non- *Salmonella* cultures produce a brick-red reaction in LIA slants.
10. All cultures that give an alkaline butt in LIA, regardless of TSI reaction, should be retained as potential *Salmonella* isolates and submitted for biochemical and serological tests. Cultures that give an acid butt in LIA and an alkaline slant and acid butt in TSI should also be considered potential *Salmonella* isolates and should be submitted for biochemical and serological tests. Cultures that give an acid butt in LIA and an acid slant and acid butt in TSI may be discarded as not being *Salmonella*. Test retained, presumed-positive TSI cultures as directed in D-11, below, to determine if they are *Salmonella* species, including *S. arizonae*. If TSI cultures fail to give typical reactions for *Salmonella* (alkaline slant and acid butt) pick additional suspicious colonies from selective medium plate not giving presumed-positive culture and inoculate TSI and LIA slants as described, above.
11. Apply biochemical and serological identification tests to:
 - a. Three presumptive TSI cultures recovered from set of plates streaked from RV medium (or SC broth for guar gum), if present, and 3 presumptive TSI agar cultures recovered from plates streaked from TT broth, if present.
 - b. If 3 presumptive-positive TSI cultures are not isolated from one set of agar plates, test other presumptive-positive TSI agar cultures, if isolated, by biochemical and serological tests. Examine a minimum of 6 TSI cultures for each 25 g analytical unit or each 375 g composite.

ANNEX E

Enumeration of *E.coli* and Coliform Count (Enumeration of *Escherichia coli* and the Coliform Bacteria (USFDA, 2001))

Conventional Method for coliforms, fecal coliforms and *E. coli*

A. Equipment and materials

1. Covered water bath, with circulating system to maintain temperature of $45.5 \pm 0.2^{\circ}\text{C}$. Water level should be above the medium in immersed tubes.
2. Immersion-type thermometer, $1\text{-}55^{\circ}\text{C}$, about 55 cm long, with 0.1°C subdivisions, certified by National Institute of Standards and Technology (NIST), or equivalent
3. Incubator, $35 \pm 1.0^{\circ}\text{C}$
4. Balance with capacity of ≥ 2 kg and sensitivity of 0.1 g
5. Blender and blender jar
6. Sterile graduated pipets, 1.0 and 10.0 mL
7. Sterile utensils for sample handling
8. Dilution bottles made of borosilicate glass, with polyethylene screw caps equipped with Teflon liners. Commercially prepared dilution bottles containing sterile Butterfield's phosphate buffer can also be used.
9. Quebec colony counter, or equivalent, with magnifying lens
10. Longwave UV light [~ 365 nm], not to exceed 6 W.
11. pH meter

B. Media and Reagents

Brilliant green lactose bile (BGLB) broth, 2% (M25)

Lauryl tryptose (LST) broth (M76)

EC broth (M49)

Levine's eosin-methylene blue (L-EMB) agar (M80)

Butterfield's phosphate-buffered water (R11) or equivalent diluent (except for shellfish)

Lauryl tryptose MUG (LST-MUG) broth (M77)

Peptone Diluent, 0.1% (R56)

MPN - Presumptive test for coliforms, fecal coliforms and *E. coli*

Weigh 50 g food into sterile high-speed blender jar. (see Chapter 1 and current FDA compliance programs for instructions on sample size and compositing) Frozen samples can be softened by storing it for ≤ 18 h at $2\text{-}5^{\circ}\text{C}$, but do not thaw. Add 450 mL of Butterfield's phosphate-buffered water and blend for 2 min. If < 50 g of sample are available, weigh portion that is equivalent to half of the sample and add sufficient volume of sterile diluent to make a 1:10 dilution. The total volume in the blender jar should completely cover the blades.

Prepare decimal dilutions with sterile Butterfield's phosphate diluent. Number of dilutions to be prepared depends on anticipated coliform density. Shake all suspensions 25 times in 30 cm arc or vortex mix for 7 s. Do not use pipets to deliver $< 10\%$ of their total volume. Transfer 1 mL portions to 3 LST tubes for each dilution for at least 3 consecutive dilutions. Hold pipet at angle so that its lower edge rests against the tube. Let pipet drain 2-3 s. Not more than 15 min should elapse from time the sample is blended until all dilutions are inoculated in appropriate media.

NOTE: Use 5-tube MPN for analysis of shellfish and shellfish harvest waters.

Incubate LST tubes at 35°C. Examine tubes and record reactions at 24 ± 2 h for gas, i.e., displacement of medium in fermentation vial or effervescence when tubes are gently agitated. Re-incubate gas-negative tubes for an additional 24 h and examine and record reactions again at 48 ± 2 h. Perform confirmed test on all presumptive positive (gas) tubes.

MPN - Confirmed test for coliforms

From each gassing LST tube, transfer a loopful of suspension to a tube of BGLB broth, avoiding pellicle if present. Incubate BGLB tubes at 35°C and examine for gas production at 48 ± 2 h. Calculate most probable number (MPN) of coliforms based on proportion of **confirmed** gassing LST tubes for 3 consecutive dilutions.

MPN - Confirmed test for fecal coliforms and *E. coli*

From each gassing LST tube from the Presumptive test, transfer a loopful of each suspension to a tube of EC broth (a sterile wooden applicator stick may also be used for these transfers). Incubate EC tubes 24 ± 2 h at 45.5 °C and examine for gas production. If negative, reincubate and examine again at 48 ± 2 h. Use results of this test to calculate fecal coliform MPN. To continue with *E. coli* analysis, proceed to Section F under Enumeration of Escheria coli and the Coliform Bacteria of the USFDA Bacteriological Analytical Manual (2001). The EC broth MPN method may be used for seawater and shellfish since it conforms to recommended procedures (1). (Caution: see Note below).

NOTE: Fecal coliform analyses are done at 45.5± 0.2°C for all foods, except for water testing and in shellfish and shellfish harvest water analysis, which uses an incubation temperature of 44.5± 0.2°C.

ANNEX F

Enumeration of Standard Plate Count

Conventional Plate Count Method (USFDA, 2001)

A. Equipment and materials

1. Work area, level table with ample surface in room that is clean, well-lighted (100 foot-candles at working surface) and well-ventilated, and reasonably free of dust and drafts. The microbial density of air in working area, measured in fallout pour plates taken during plating, should not exceed 15 colonies/plate during 15 min exposure.
2. Storage space, free of dust and insects and adequate for protection of equipment and supplies
3. Petri dishes, glass or plastic (at least 15 x 90 mm)
4. Pipets with pipet aids (no mouth pipetting) or pipettors, 1, 5, and 10 ml, graduated in 0.1 ml units
5. Dilution bottles, 6 oz (160 ml), borosilicate-resistant glass, with rubber stoppers or plastic screw caps
6. Pipet and petri dish containers, adequate for protection
7. Circulating water bath, for tempering agar, thermostatically controlled to $45 \pm 1^\circ\text{C}$
8. Incubator, $35 \pm 1^\circ\text{C}$; milk, $32 \pm 1^\circ\text{C}$
9. Colony counter, dark-field, Quebec, or equivalent, with suitable light source and grid plate
10. Tally register
11. Dilution blanks, 90 ± 1 ml Butterfield's phosphate-buffered dilution water (R11); milk, 99 ± 2 ml
12. Plate count agar (standard methods) (M124)
13. Refrigerator, to cool and maintain samples at $0-5^\circ\text{C}$; milk, $0-4.4^\circ\text{C}$
14. Freezer, to maintain frozen samples from -15 to -20°C
15. Thermometers (mercury) appropriate range; accuracy checked with a thermometer certified by the National Institute of Standards and Technology (NIST)

B. Procedure for analysis of frozen, chilled, precooked, or prepared foods

Using separate sterile pipets, prepare decimal dilutions of 10^{-2} , 10^{-3} , 10^{-4} , and others as appropriate, of food homogenate ([see Chapter 1](#) for sample preparation) by transferring 10 ml of previous dilution to 90 ml of diluent. Avoid sampling foam. Shake all dilutions 25 times in 30 cm (1 ft) arc within 7 s. Pipet 1 ml of each dilution into separate, duplicate, appropriately marked petri dishes. Reshake dilution bottle 25 times in 30 cm arc within 7 s if it stands more than 3 min before it is pipetted into petri dish. Add 12-15 ml plate count agar (cooled to $45 \pm 1^\circ\text{C}$) to each plate within 15 min of original dilution. Pour agar and dilution water control plates for each series of samples. Immediately mix sample dilutions and agar medium thoroughly and uniformly by alternate rotation and back-and-forth motion of plates on flat level surface. Let agar solidify. Invert solidified petri dishes, and incubate promptly for 48 ± 2 h at 35°C . Do not stack plates when pouring agar or when agar is solidifying.