



***Live and Active Culture Yogurt  
Seal Program***

Procedures and Guidelines

*(Updated April 8, 2008)*

National Yogurt Association  
2000 Corporate Ridge, Suite 1000  
McLean, VA 22102  
703.821.0770

## NATIONAL YOGURT ASSOCIATION SEAL APPLICATION RULES AND PROCEDURES

This document, including appendices, sets forth the rules and procedures for obtaining permission to use the National Yogurt Association (NYA) Seal for refrigerated cup and/or frozen yogurt products containing live and active cultures. The rules and procedures may be modified from time to time by the NYA Board of Directors.

### I. ELIGIBILITY

A. Any company which produces and/or distributes yogurt (refrigerated cup and/or frozen) in the United States, whether or not a member of the NYA, may apply to use the NYA Seal on product labels or in labeling or advertising.

B. A separate application must be submitted for each product for which use of the NYA Seal is sought. For purposes of this program, "product" is defined as a brand of yogurt of a particular type or form including an aggregation of different flavors of a type of form. By way of example, each of the following is considered a separate product:

Nonfat yogurt – fruit on the bottom (all flavors)  
Low fat yogurt – fruit on the bottom (all flavors)  
Custard style yogurt (all flavors)  
Low fat frozen yogurt (all flavors)

Yogurt sweetened with aspartame (or any other similar non-nutritive sweetener) and yogurt sweetened with a nutritive sweetener, such as sucrose, are also considered separate products.

C. If a company submits test results for the same product, as defined in Section I.B., which is sold under more than one brand name, the application must contain a list of all the brand names under which the product is sold.

D. A company which wishes to apply to use the Seal must submit a signed application form with the specified information and fee, to the National Yogurt Association, 2000 Corporate Ridge, Suite 1000, McLean, VA 22102; ATTN: Seal Program.

- E. Applications must be accompanied by the fee specified in Section II.A.

## II. FEES

- A. A company which produces and/or distributes yogurt products may apply to use the Seal at a cost of \$2,500 per application. So, for example, if a company wants to use the Seal on four different types of yogurt products (as defined in I.B.) the total fee would be \$10,000. The fee, which is non-refundable, is for the sole purpose of offsetting the costs of the administration of the program.
- B. The dues of voting Members of the NYA include funds to cover an unlimited number of applications per 12-month period. The dues of active non-voting Members of the NYA include funds to cover ten applications per 12-month period. Additional applications must be accompanied by the fee set forth in paragraph A.

## III. CONTENT OF APPLICATION

An application shall consist of the following:

- A. A completed and signed Application Form for each type of yogurt product on which the requestor intends to use the Seal.
- B. The results of the analytical tests (conducted in accordance with the protocol set forth in Appendix A, including full reports of analytical procedures, signed worksheets, etc.) which establish the presence of live and active yogurt cultures in the product. The analytical tests must be conducted at independent laboratories (that is, not in the laboratories of the company which is applying to use the Seal). A list of independent laboratories known to be experienced in conducting tests in accordance with the requisite protocols is found in Appendix C. Other laboratories may be equally qualified to perform the analytical work.
- C. A check payable to the NYA for the appropriate fee, if a fee is required. See Section II. of this document.

#### IV. TEST PROTOCOLS

- A. See Appendix A for specific protocols. In general, the company shall provide, to the independent laboratory it has selected, three samples for the activity test and three samples for the culture test. For frozen yogurt products, the titratable acidity shall also be determined and the applicant must certify that each product under review contains a yogurt component that was produced through fermentation. The independent laboratory shall analyze two (2) of the samples for each test to determine if they meet the requirements specified in Appendix A.
- B. If the two samples for each test pass, the product will be considered as meeting the Seal Program requirements.
- C. If one of the two samples does not pass any one of the tests, the laboratory shall test the third sample.
  - 1. If the third sample passes, the product satisfies the requirements for use of the Seal.
  - 2. If the third sample fails, the company must begin the entire testing process again by taking three new samples from a single production run.
- D. If both of the original samples fail any one of the tests, a company shall proceed as if the third sample failed, and thus follow the procedures specified in Section IV.C.2. Reports submitted with an application shall include all results, including results of analyses on samples that did not pass any one of the tests.

#### V. AWARD/DENIAL OF SEAL

- A. Decisions regarding whether to award or deny use of the Seal shall be made by the NYA Seal Program Committee, which consists of the President of NYA (Chairperson of the Committee) and two other representatives from the staff of NYA. The decision is based solely on whether: (1) the application submitted is complete; and (2) complies with the specified requirements for use of the Seal. The Committee, in its discretion, may ask the applicant for additional information.
- B. The Seal Program Committee may consult with the Regulatory and Public Affairs Committee of the Association. If the product being

reviewed involves an NYA member, the member's representative on the Committee is not permitted to advise on that product.

- C. Decisions on whether to award or deny use of the Seal will be made within 10 working days from the date a completed application is received. Applicants will be notified in writing promptly of the Seal Program Committee's decision.

## VI. APPEALABILITY OF DECISION

- A. If an applicant's request for use of the Seal is denied, the applicant may request a hearing before the Seal Program Committee. The hearing will be held within 20 working days from the date of the request, unless the applicant and the Seal Program Committee agree to extend the time period.
- B. The hearing will be held in Washington, D.C. area. The hearing may be by telephone, if agreed to by the applicant.
- C. The hearing is informal in nature. The applicant may present written or oral testimony and/or argument and may be represented by counsel. A memorandum of the hearing will be made by the chairman of the Seal Program Committee and provided to the applicant.
- D. The Seal Program Committee will render a decision within five working days following a hearing. All decisions made by the Committee on appeal are final.

## VII. ANNUAL RENEWAL/RECERTIFICATION

- A. September 30 is the annual renewal deadline for all products utilizing the Seal. Applications or renewals received on or before this date during the same calendar year, that are subsequently approved, will be valid until September 30 of the following year.
- B. Continued use of the Seal will be granted each year upon submission of a renewal application certifying that a material change (e.g. change in cultures used or a significant change in manufacturing processes) has not occurred in the manufacture of the product or upon provision to the Seal Program Committee of current information which demonstrates that the product still conforms to the required criteria. Where new information is provided, results of the analysis in accordance with Appendix A also must be submitted.

- C. A material change in the product or its method of manufacture that reasonably could affect compliance with the requirements will cause the right to use the Seal to end immediately, unless a new application has been approved.
- D. When a material change has occurred in a product, the renewal application must be accompanied by a non-refundable fee of \$2,500 for that product. If there are no material changes in the product, the renewal fee is \$2,000 for that product. Active, non-voting NYA members are allowed up to 10 initial and/or renewal applications per 12-month period (September to September). Voting members' dues include funds for an unlimited number of initial and/or renewal applications per 12-month period.
- E. Tests conducted to determine eligibility for the Seal are valid for three years (unless testing is otherwise required under the Seal criteria). At the end of the third year, a company must submit, along with its application for renewal, test results performed in the previous three months demonstrating that the product, for which the renewal application is submitted, still meets NYA Seal criteria.
- F. It is the responsibility of any company that wishes to continue to use the Seal to ensure compliance with the renewal provisions under this section.

#### VIII. USE OF NYA SEAL

- A. The NYA Seal is as follows:



- B. NYA recommends that the logo portion of the Seal appear on the Principal Display Panel of the product's label printed with a positive image. The acceptable minimum size is when the "L" in the "Live" of the logo equals 1/16<sup>th</sup> of an inch.
- C. NYA recommends that the logo be as close as possible to the bottom left hand corner of the Principal Display Panel. The asterisked

statement should be as close as possible to the logo but it may appear anywhere on the label.

- D. Color: For NYA Seals on packages, NYA recommends Process Magenta or Process Blue on packages with 4-color processing. On non – 4 – color processing, NYA recommends the use of the darkest or most prominent color of the package graphics. On labeling and in advertising, any suitable color may be used.
- E. A company may use the Seal on the product's label, labeling, or advertising or in other promotional materials.

## **APPENDICES**

- A. NYA CRITERIA, SAMPLING AND ANALYTICAL PROCEDURES
- B. INTERNATIONAL DAIRY FEDERATION STANDARDS FOR YOGURT: *ENUMERATION OF CHARACTERISTIC MICROORGANISMS AND TITRATABLE ACIDITY*
- C. LIST OF INDEPENDENT LABORATORIES
- D. NYA LABORATORY REPORT FORM
- E. NYA LIVE AND ACTIVE CULTURES SEAL APPLICATION

## NATIONAL YOGURT ASSOCIATION CRITERIA FOR LIVE AND ACTIVE CULTURE YOGURT

Live and active culture yogurt (refrigerated cup and frozen yogurt) is the food produced by culturing Grade A dairy ingredients with a characterizing bacterial culture in accordance with the standards of identity for yogurt (21 C.F.R. S 131.200), low fat yogurt (21 C.F.R. S 131.203), and nonfat yogurt (21 C.F.R. S 131.206). In addition to the use of the bacterial cultures required by the referenced federal standards of identity and by these NYA criteria, live and active culture yogurt may contain other safe and suitable food grade bacterial cultures. Declaration of the presence of cultures on the label of live and active culture yogurt is optional.

Heat treatment of live and active yogurt is inconsistent with the maintenance of live and active cultures in the product; accordingly, heat treatment which is intended to kill the live and active organisms shall not be undertaken after fermentation. Likewise, manufacturers of live and active culture yogurt should undertake their best efforts to ensure that distribution practices, code dates, and handling instructions are conducive to the maintenance of live and active cultures.

In order to meet these NYA criteria, live and active culture yogurt must satisfy each of these requirements:

1. The product must be fermented with both *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*.
2. For refrigerated yogurt, the total viable count at the time of manufacture will be  $10^8$  CFU per gram. In the case of frozen yogurt, the total viable count at the time of manufacture will be  $10^7$  CFU per gram.
3. The cultures must be active at the end of the stated shelf - life as determined by the activity test described in the "Sampling and Analytical Procedures." Compliance with this requirement shall be determined by meeting the criteria for the activity test on two of the three representative samples of yogurt which have been stored at temperatures between 32 °F and 45 °F for refrigerated cup yogurt and at temperatures of 0 °F or colder for frozen yogurt for the entire stated shelf-life of the product. The activity test is met if there is an increase of 1(one) log during fermentation.

4. In the case of frozen yogurt, the product shall have a total titratable acidity expressed as lactic acid of at least 0.3 % at all times. In addition, the applicant must certify in writing that at least 0.15 % titratable acidity in the product was derived from fermentation by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*.

### SAMPLING AND ANALYTICAL PROCEDURES

The applicant should submit three samples representing a single line of product, ideally taken from the beginning, middle and end of a single manufacturing run, plus three additional samples of the same product line that is at the end of the determined shelf-life date<sup>1</sup>, that demonstrates that the yogurt has met the standard. The samples will be analyzed according to the following procedures:

#### Refrigerated Yogurt

- (a) Total viable yogurt counts will be enumerated by the attached IDF procedure (Appendix B). The total viable count will be reported on the NYA Laboratory Report Form (see Appendix D). The total viable count is the sum of colony forming units of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* per gram of the product.
- (b) At the end of the stated shelf-life designated by the applicant, activity of the culture will be reported for at least two of the three random samples on the NYA Laboratory Report Form.

The activity test is carried out by pasteurizing 12 % solids non-fat dry milk at 198 °F for seven minutes, cooling to 110 °F, adding 3 % inoculum of the material under test and fermenting at 110 °F for 4 hours. The total yogurt organisms in the inoculated milk substrate are to be enumerated both before and after fermentation by IDF methodology (see Appendix B; International IDF Standard 117A: 1988).

The activity test will be reported as log increase in yogurt organisms (CFU/g) following fermentation of the defined substrate under the standard condition at the end of the stated shelf life.

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<sup>1</sup> The shelf-life date, whether appearing on the product label or not, shall be determined by the manufacturer according to standard company practice.

## Frozen Yogurt

- (a) The titratable acidity (TA) of samples, one each representing the beginning, middle and end of a manufacturing run, will be determined as per the IDF procedure in Appendix B and reported on the NYA Laboratory Report Form. The TA must be at least 0.3 % at all times during manufacture.
- (b) Total viable yogurt counts will be enumerated by the IDF procedure in Appendix B. The total viable count will be reported on the NYA Laboratory Report Form. The total viable count is the sum of colony forming units of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* per gram of the product.
- (c) At the end of the stated shelf life designated by the applicant, activity of the culture will be reported for at least two of the three random samples on the Laboratory Report Form.

The activity test is carried out by pasteurizing 12 % solids non-fat dry milk at 198 °F for 7 minutes, cooling to 110 °F, adding 3 % inoculum of the material under test and fermenting at 110 °F for 4 hours. The total yogurt organisms in the inoculated milk substrate are to be enumerated both before and after fermentation by the IDF methodology.

The activity will be reported as log increase in yogurt organisms (CFU/g) following fermentation of the defined substrate under the standard conditions at the end of the stated shelf life.



# INTERNATIONAL IDF STANDARD 117A:1988

IDF - SQUARE VEIGOTE 41, B-1040 BRUSSELS (BELGIUM)

Price: 300 Belgian Francs

## YOGURT ENUMERATION OF CHARACTERISTIC MICROORGANISMS COLONY COUNT TECHNIQUE AT 37°C

### 1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a method for the enumeration of the characteristic microorganisms in yogurt by means of the colony count technique at 37°C.

The method is applicable to yogurts where both characteristic microorganisms are present and viable.

### 2 REFERENCES

*IDF Standard 50B : 1985, Milk & milk products - Methods of sampling.*

*IDF Standard 122A : 1988, Milk & milk products - Preparation of samples and dilutions for microbiological examination.*

### 3 DEFINITION

For the purposes of this standard, the following definitions for the characteristic microorganisms apply:

**3.1 *Lactobacillus bulgaricus*:** a thermophilic microorganism that forms lenticular often sharp-shaped colonies, of diameter 1 to 3 mm on acidified MRS medium (according to De Man, Rogosa & Sharpe, see 12.1) under the conditions specified in this International Standard.

Microscopic appearance: rods, generally short, but sometimes in longer forms. They are non-spore forming, Gram-positive, non-motile and catalase-negative.

**3.2 *Streptococcus thermophilus*:** a thermophilic microorganism that forms lenticular colonies of diameter 1 to 2 mm on M17 medium (according to Terzaghi, see 12.2) under the conditions specified in this International Standard.

Microscopic appearance: spherical or ovoid cells (0.7 - 0.9 µm in diameter) in pairs or in long chains. They are Gram-positive and catalase-negative.

### 4 PRINCIPLE

4.1 Inoculation of decimal dilutions of the sample into:

a) acidified MRS medium, followed by anaerobic incubation at 37°C for 72 h, for the count of *L. bulgaricus*;

b) M17 medium, followed by aerobic incubation at 37°C for 48 h, for the count of *S. thermophilus*.

4.2 Counting of colonies and confirmation by means of appropriate tests.

4.3 Calculation of the number of characteristic microorganisms per gram of sample from the number of colonies obtained on plates chosen at dilution levels so as to give a significant result.

### 5 DILUENTS, CULTURE MEDIA AND REAGENTS

#### 5.1 Basic materials

In order to improve the reproducibility of the results, it is recommended that, for the preparation of the diluent, dehydrated basic components or a dehydrated complete preparation should be used. The manufacturer's instructions shall be rigorously followed.

Chemical reagents shall be of recognized analytical quality. The water used shall be water distilled from glass apparatus, or deionized water. It shall be free from substances that might influence the growth of microorganisms under test conditions. This shall be periodically checked, particularly in the case of deionized water.

*Note.- Tests to determine the suitability of water for microbiological applications have been published in "Standard methods for the examination of dairy products", 15th Edition 1984, Ed. E.H. Marth, American Public Health Association, Washington DC, USA.*

Solutions of sodium hydroxide or hydrochloric acid (approximately 0.1 mol/litre) shall be used to adjust the pH of diluents, unless otherwise specified.

This standard was developed by a joint IDF/ISO/AOAC Group of Experts (Group E44 - Characteristic microorganisms in yogurt - Chairman: Prof. R. Negri, Italy) and was approved for publication as a final standard at the IDF Annual Sessions in Helsinki, Finland, in September 1987 (report E-Doc 284).

The corresponding text issued by ISO is ISO/DIS 7889.

This standard supersedes Standard 117 : 1983, which is thus withdrawn.

## 5.2 Diluent

### Composition

Peptone 1 (tryptic digest of casein)	0.5 g
Peptone 2 (tryptic digest of meat)	0.5 g
Water	1 000 ml

### Preparation

Dissolve the peptones in water. Distribute the solution in 100 ml portions in bottles of capacity 150 ml. Sterilize at 121°C for 15 min.

*Note.* - Peptone mixtures of this type (peptone 1 and peptone 2) are available commercially, for example, the so-called "polypeptones".

## 5.3 Culture media

### 5.3.1 Acidified MRS medium (see 12.1)

#### Composition

Peptone 1	10 g
Meat extract	10 g
Yeast extract (dried)	5 g
Glucose (C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> )	20 g
Tween 80 (sorbitan mono-oleate)	1 ml
Dipotassium hydrogenorthophosphate (K <sub>2</sub> HPO <sub>4</sub> )	2 g
Sodium acetate trihydrate (CH <sub>3</sub> CO <sub>2</sub> Na.3H <sub>2</sub> O)	5 g
Diammonium citrate [C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> (NH <sub>4</sub> ) <sub>2</sub> ]	2 g
Magnesium sulphate heptahydrate (MgSO <sub>4</sub> .7H <sub>2</sub> O)	0.2 g
Manganese sulphate tetrahydrate (MnSO <sub>4</sub> .4H <sub>2</sub> O)	0.05 g
Agar (according to the manufacturer's instructions; see also 12.1)	9 to 18 g
Water	1 000 ml

#### Preparation

Dissolve the components in boiling water. Cool to 50°C and add acetic acid (5.4.3) to adjust the pH, checking by means of a pH meter (6.1.8), so that after sterilization it will be 5.4 at 25°C. Distribute the medium in portions of 100 ml into bottles of capacity 150 ml or in portions of 200 ml into bottles of capacity 250 ml. Sterilize at 121°C for 15 min.

*Note.* - Comparative tests have shown that commercially available MRS media may give counts that are lower than those given by the MRS medium prepared in accordance with this International Standard. Therefore, if used, the former should be checked against the medium prepared according to this International Standard.

Before beginning the bacteriological examination, completely melt the required amount of medium in a boiling water bath or by steaming in a partially closed container, then cool it in the water bath (6.1.7).

*Note.* - It is recommended, in order to check the temperature of the agar, to place a thermometer in a separate container identical to those used for the medium, filled with the same volume of agar solution at the same concentration. This temperature control solution should be exposed to the same heating and cooling operations as the medium itself.

### 5.3.2 M17 medium (see 12.2)

#### 5.3.2.1 Basic medium

##### Composition

Peptone 1 (tryptic digest of casein)	2.50 g
Peptone 2 (peptic digest of meat)	2.50 g
Peptone 3 (papain digest of soya)	5.00 g
Yeast extract (dried)	2.50 g
Meat extract	5.00 g
β-glycerophosphate (disodium salt)(C <sub>3</sub> H <sub>7</sub> O <sub>6</sub> PN <sub>2</sub> )	19.00 g
Magnesium sulfate heptahydrate(MgSO <sub>4</sub> .7H <sub>2</sub> O)	0.25 g
Ascorbic acid (C <sub>6</sub> H <sub>8</sub> O <sub>6</sub> )	0.50 g
Agar (according to the manufacturer's instructions)	9 to 18 g
Water	950 ml

### Preparation

Dissolve the components in boiling water. Cool to 50°C and adjust the pH, using the reagents (5.4), checking by means of a pH meter (6.1.8), so that after sterilization it will be 7.1 to 7.2 at 25°C. Transfer the medium in portions of 95 ml into bottles of capacity 150 ml. Sterilize at 121°C for 15 min.

*Note.* - Complete M17 media are commercially available but as in the case of commercially available MRS media, the results obtained may differ significantly from one supplier to the other. Therefore they should be checked against M17 medium prepared according to this International Standard.

### 5.3.2.2 Lactose solution

#### Composition

Lactose (C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )	10 g
Water	100 ml

#### Preparation

Dissolve the lactose in the water; sterilize at 121°C for 15 min.

### 5.3.2.3 Complete medium

#### Composition

Basic medium (5.3.2.1)	95 ml
Lactose solution (5.3.2.2)	5 ml

#### Preparation

Immediately before use melt the basic medium (5.3.2.1) in a boiling water bath and cool to 48-50°C. Heat the lactose solution (5.3.2.2) to 48-50°C. Add the lactose solution to the basic medium and mix by swirling.

Cool the medium in the water bath (6.1.7).

## 5.4 Reagents for pH adjustment.

5.4.1 Sodium hydroxide (NaOH), approx. 0.1 mol/l solution.

5.4.2 Hydrochloric acid (HCl), approx. 0.1 mol/l solution.

5.4.3 Acetic acid (CH<sub>3</sub>COOH), 100% (glacial).

## 6 APPARATUS AND GLASSWARE

6.1 Besides the usual microbiological laboratory equipment, the equipment required for the preparation of test samples and dilutions as specified in IDF Standard 122A, and in particular:

6.1.1 Incubator 37 ± 1°C.

6.1.2 Anaerobic incubator, capable of being controlled at 37 ± 1°C, or anaerobic culture jars, providing an atmosphere of 90% nitrogen and 10% carbon dioxide.

6.1.3 Blender, either a peristaltic-type blender (stomacher) with sterile plastic containers, or a rotary blender capable of operating at a minimum rotational frequency of 20 000 min<sup>-1</sup>, with sterile glass or metal containers of appropriate capacity.

6.1.4 Test tube agitator (for example, vortex mixer).

6.1.5 Colony counting equipment consisting of an illuminated base with a dark background fitted with a magnifying lens to be used at a magnification of 1.5 x and a mechanical or electronic digital counter.

6.1.6 Lens, magnification 8 - 10 x.

6.1.7 Water bath, capable of being controlled at 45 ± 1°C.

6.1.8 pH meter, with temperature compensation, accurate to ± 0.1 pH unit at 25°C.

6.1.9 Dilution flasks, 150-250 ml capacity, and test tubes 18 x 180 mm, with suitable seal cap or stopper made of rubber or a synthetic material.

6.1.10 Flasks or bottles, 150-250 ml capacity, and test tubes, about 20 ml capacity, to hold the culture medium.

6.1.11 Graduated pipettes, calibrated to the tip to deliver 1 ml ± 0,02 ml or 10 ml ± 0,2 ml or 11 ml ± 0,2 ml.

6.1.12 Petri dishes, of clear uncoloured glass, the bottom having an internal diameter of about 90 mm. The internal depth should be 10 mm minimum. The bottom shall have no irregularities that may interfere with counting colonies.

*Note.* - Presterilized pipettes and Petri dishes made of synthetic materials may be used instead of the glass pipettes and Petri dishes.

6.1.13 Glass or metal spatula.

6.2 Sterilization of equipment that will come into contact with the test sample, the diluent, the dilutions or the culture medium, shall be carried out in accordance with clause 6.1 of IDF Standard 122A.

## 7 SAMPLING

See IDF Standard 50B : 1985.

## 8 PROCEDURE

### 8.1 Preparation of the test sample and test portion

#### Precaution

Before opening the yogurt container, clean the external surface immediately surrounding the area from which the sample is to be taken, in order to remove any material that might contaminate the sample. The area may be swabbed with 70% (V/V) ethanol to prevent further contamination. Open the container aseptically.

*Note.* - During this stage, it is important to obtain not only a homogeneous dilution, but also a fragmentation of the chains of streptococci and lactobacilli into individual cells or short chains, so that the results, expressed as a total viable count per gram of product, are reproducible and representative.

#### 8.1.1 Non-fruit yogurts

Carefully mix the contents of the yogurt container using a sterile spatula (6.1.13). Weigh  $10 \pm 0.1$  g of the sample in a suitable container [for example a 200 ml round-bottom centrifuge tube made of strengthened glass, or the bowl of the rotary blender, or the pastic container of the peristaltic blender (6.1.3)].

#### 8.1.2 Fruit yogurts

Blend the complete contents of the yogurt container for 1 min, using the blender (6.1.3). Weigh  $10 \pm 0.1$  g of the sample as in 8.1.1.

#### 8.2 Microscopic examination

Carry out a preliminary microscopic examination of several fields of a smear of the yogurt sample, previously dyed with methylene blue (for example a 6 g/litre methylene blue ethanolic solution), to estimate the density of the two bacterial types, cocci and rods, and to select the proper range of dilutions to be used for the count of each type.

### 8.3 Preparation of primary dilution

*Note.* - The operations described in 8.3 to 8.5.4 should not be carried out in direct sunlight.

Add the diluent (5.2) to the test portion (8.1.1 or 8.1.2) until the mass of test portion and diluent is 50 g.

Blend for 1 min, using the blender (6.1.3).

Make up to 100 g with the same diluent. A  $10^{-1}$  dilution is thus obtained.

### 8.4 Preparation of decimal dilutions

Distribute the diluent (5.2) into 9 ml portions in test tubes (6.1.10) using a sterile 10 ml pipette. Add 1 ml of the primary dilution to the first tube. Mix for 10 s on a test tube agitator (6.1.4). Transfer 1 ml of this to the second tube so as to obtain

a  $10^{-2}$  dilution. Repeat this operation until the required dilution series is obtained, using a fresh pipette for each dilution step.

*Note.* - Do not dip the pipette more than 1 cm below the surface of the liquid. Avoid filling the pipette with air bubbles. When transferring do not dip the pipette in the new diluent.

## 8.5 Inoculation and Incubation

*Note.* - The time elapsing between the inoculation of the dilutions and the pouring into Petri dishes (8.5.2 and 8.5.3) should not exceed 15 min.

8.5.1 Proceeding in duplicate pairs (for both *L. Bulgaricus* and *S. thermophilus*), transfer, using a sterile pipette, 1 ml of each dilution into Petri dishes (6.1.12).

8.5.2 For *L. bulgaricus*, pour 12-15 ml of acidified MRS medium (5.3.1) maintained at  $45 \pm 1^\circ\text{C}$  on a water bath (6.1.7) into each Petri dish.

8.5.3 For *S. thermophilus*, pour 12-15 ml of M17 medium (5.3.2) maintained at  $45 \pm 1^\circ\text{C}$  on a water bath (6.1.7) into each Petri dish.

8.5.4 Carefully mix, immediately after pouring, the inoculum with the medium by rotating the Petri dishes and allow the mixture to solidify by leaving the Petri dishes to stand on a cool horizontal surface.

8.5.5 Incubate the prepared dishes in an inverted position. Stack not more than six high. Stacks of dishes should be separated from one another and from the walls and top of the incubator.

8.5.5.1 Incubate the plates to be used for the enumeration of *L. bulgaricus* at  $37 \pm 1^\circ\text{C}$  for 72 h in the anaerobic incubator or anaerobic culture jar (6.1.2).

8.5.5.2 Incubate the plates to be used for the enumeration of *S. thermophilus* at  $37 \pm 1^\circ\text{C}$  for 48 h.

## 8.6 Counting of colonies

8.6.1 After the specified period of incubation (8.5.5.1 and 8.5.5.2), count the colonies, showing the features of each characteristic micro-organism (3.1 and 3.2) on plates having between 10 and 300 colonies.

Examine the plates, in subdued light. To facilitate counting, a suitable colony counting equipment may be used (6.1.5). Avoid mistaking particles of undissolved sample or precipitated matter in dishes for pinpoint colonies. Examine doubtful objects carefully, using a lens of higher magnifications where required, to distinguish colonies from foreign matter.

## 8.7 Confirmation

Select colonies from the plates used for counting such that the number taken is equal to the square root of the total colony count. Stain these colonies using the Gram method and confirm that they are non-spore forming, Gram-positive, catalase-negative rods in the case of those grown on the MRS medium, and Gram-positive, catalase-negative chains of cocci or diplococci in the case of those grown on the M17 medium.

## 9 EXPRESSION OF RESULTS

### 9.1 Method of calculation

9.1.1 Use counts from plates containing between 10 and 300 colonies as obtained in 8.6.

9.1.2 The number of each characteristic micro-organism as per gram is equal to

$$\frac{\Sigma C}{(n_1 + 0,1 n_2) d}$$

where

$\Sigma C$  is the sum of colonies counted on the plates as in 9.1.1;  
 $n_1$  is the number of plates counted in the lower dilution;  
 $n_2$  is the number of plates counted in the higher dilution;  
 $d$  is the value corresponding with the dilution from which the first counts were obtained.

*Note.* - If there are more than two countable dilutions, the formula should be modified to take the further dilution into account.

Thus for 3 dilutions

$$\frac{\Sigma C}{(n_1 + 0,1 n_2 + 0,01 n_3) d}$$

9.1.3 Round the result obtained in 9.1.2 to two significant digits. For a three-digit number, round the third digit to the nearest zero. If the third digit is 5, round to the digit below in case the first two digits are an even number, and to the digit above in case the first two digits are an odd number.

For example:

for	234	round to	230
	235		240
	225		220
	245		240

9.1.4 If there are only counts less than 10, report the number of micro-organisms per gram as "less than  $10 \times 1/d$ "; "d" being the value corresponding with the lowest dilution.

9.1.5 If there are only counts exceeding 300, calculate an estimated count from dishes having a count nearest 300 colonies and multiply with the reciprocal of the value corresponding with the highest dilution. Report as the "lower case estimated number of micro-organisms per gram".

9.1.6 The result shall be expressed as a number from 1,0 to 9,9 multiplied by the appropriate power of 10.

9.1.7 The total number of characteristic micro-organisms per gram of yogurt is equal to:

$$N_L + N_S$$

where

$N_L$  is the number of *L. bulgaricus* per gram, calculated in 9.1.2;

$N_S$  is the number of *S. thermophilus* per gram, calculated in 9.1.2.

## 9.2 Example of calculation

Assume that a *L. bulgaricus* count gave the following results (two Petri dishes per dilution were incubated):

$10^{-5}$  dilution: 295 and 245 colonies;

$10^{-6}$  dilution: 33 and 40 colonies;

$$\begin{aligned} \text{then } \frac{\Sigma C}{(n_1 + 0,1 n_2) d} &= \frac{295 + 245 + 33 + 40}{(2 + 0,1 \times 2) 10^{-5}} \\ &= \frac{613}{2,2 \times 10^{-5}} = 278,6 \times 10^6 \end{aligned}$$

In accordance with 9.1.3 this equals to  $280 \times 10^6$ . The estimated number of *L. bulgaricus* is therefore:  $2,8 \times 10^7$ /g of yogurt.

Similarly, for *S. thermophilus*, an estimated number of  $4,9 \times 10^6$ /g of yogurt was obtained.

Thus the total number of characteristic micro-organisms is equal to:

$$(2,8 \times 10^7) + (4,9 \times 10^6) = 5,18 \times 10^7$$

which, when rounded in accordance with 9.1.3 gives:

$$5,2 \times 10^7 \text{ per gram of yogurt}$$

## 9.3 Precision

Experience indicates that if the higher of two independent tests on the same sample frequently exceeds the lower by 30%, the

analyst should examine his procedures to determine sources of error.

## 10 TEST REPORT

The test report shall show the method used, the results obtained and the form in which it is expressed. It shall also mention any operating details not specified in this International Standard, or regarded as optional, as well as any particular phenomena observed during the examination that may have influenced the results.

The report shall include all details required for the complete identification of the sample.

## 11 NOTES ON PROCEDURE

### 11.1 Selectivity of the culture media

Neither of the two recommended culture media (acidified MRS and M17) is completely selective. Most of the *S. thermophilus* strains do not form visible colonies on the acidified MRS medium in dilutions normally used for the count of *L. bulgaricus*. However, when the number of lactobacilli in the sample of yogurt is considerably lower than the number of streptococci, low dilutions have to be used for the count of *L. bulgaricus*. Under these conditions, some *S. thermophilus* may form very small or pinpoint colonies on the acidified MRS plate; these colonies can easily be differentiated with the naked eye, from the *L. bulgaricus* colonies, the latter being of larger size.

On the other hand, most *L. bulgaricus* strains do not form visible colonies on M17 plates with dilutions normally used for counting *S. thermophilus*. This confirms earlier findings (see 12.3).

Some strains of *L. bulgaricus* may however form small pinpoint colonies on the M17 medium, especially with samples of yogurt presenting a much higher number of lactobacilli compared to the number of streptococci. These small rough colonies usually have a woolly or fleecy appearance and they can easily be distinguished with the naked eye (and still better with a magnifying lens) from the smooth lenticular colonies of *S. thermophilus* which are of larger size.

### 11.2 Recommended culture media

The M17 and acidified MRS culture media were chosen on the ground of a comprehensive interlaboratory test that was carried out on the suitability of the following culture media: skim milk, MRS and M17 media acidified at pH 5,4, Lee's medium (13.4), LAB medium (13.5), LS-differential medium (13.6).

## 12. BIBLIOGRAPHY

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# PROVISIONAL IDF STANDARD 150:1991

IDF - SQUARE VERGOTE 41, B-1040 BRUSSELS (BELGIUM)

Price: 250 Belgian Francs

## Yogurt

# DETERMINATION OF TITRATABLE ACIDITY POTENTIOMETRIC METHOD

### 1 SCOPE

This International Standard specifies a potentiometric method for the determination of the titratable acidity of natural yogurt, flavoured, sweetened yogurt and fruit yogurt.

### 2 REFERENCE

IDF Standard 50B:1985 - Milk and milk products - Methods of sampling.

### 3 DEFINITION

For the purposes of this International Standard the following definition applies.

**titratable acidity of yogurt:** The number of millilitres of 0,1 mol/l sodium hydroxide solution required to titrate a quantity of yogurt to the pH of 8,30.

It is conventionally expressed as grams of lactic acid per 100 g of product.

### 4 PRINCIPLE

Suspension of a test portion in water. Potentiometric titration with 0,1 mol/l sodium hydroxide solution to the pH of 8,30.

### 5 REAGENT

The reagent shall be of recognized analytical quality. Water shall be distilled or deionized water, freed from carbon dioxide by boiling for 10 min before use.

**5.1 Sodium hydroxide, standard volumetric solution,**  $c(\text{NaOH}) = 0,1 \pm 0,0002$  mol/l, carbonate free.

Protect this solution against absorption of carbon dioxide.

### 6 APPARATUS

Usual laboratory equipment, and in particular:

**6.1 Analytical balance.**

**6.2 pH meter,** equipped with a measuring and a reference electrode, calibrated by means of two buffer solutions of pH approximately 7 and 9 respectively, known to within  $\pm 0,01$  pH unit.

**6.3 Spoon or spatula.**

### 7 SAMPLING

See IDF Standard 50B:1985.

### 8 PREPARATION OF TEST SAMPLE

#### 8.1 Natural yogurt; flavoured, sweetened yogurt

Bring the sample to a temperature of 20 to 25°C. Mix the sample carefully by means of a spoon or spatula using a rotary motion which passes from the lower layers to the surface layers of the sample so as to displace and mix them well.

#### 8.2 Fruit yogurt

Bring the sample to a temperature of 20 to 25°C. Homogenize it using an appropriate device, in order to facilitate the grinding and dispersion of the fruit.

### 9 PROCEDURE

#### 9.1 Test portion

Weigh approximately 10 g of the prepared test sample (8), to the nearest 10 mg, into a beaker of capacity 50 ml. Add approximately 10 ml of water and mix.

This standard was developed by a joint IDF/ISO/AOAC Group of Experts (E5 Water content of milk and milk products - Chairman: G. Steiger, CH) and was approved for publication as a provisional standard at the IDF Annual Sessions in Toronto, Canada, October 1990 (report E-Doc 436). The corresponding ISO text has not yet been issued.

The present standard should be considered as a 'Provisional Standard' (hence the green paper). The method given, although considered as the most suitable for the time being, is still subject to change or nullification in the light of any further experience. Information on the use of this standard in practice would be appreciated. Comments are invited by December 1992.

## 9.2 Determination

9.2.1 Insert the electrodes of the pH meter (6.2) into the suspension (9.1) and ensure that they are properly immersed.

9.2.2 Titrate the contents of the beaker, whilst stirring, using the sodium hydroxide solution (5.1), to the pH of 8.30.

Record the volume, in millilitres, of sodium hydroxide solution used, to the nearest 0,05 ml.

## 10 EXPRESSION OF RESULTS

### 10.1 Method of calculation

The titratable acidity, expressed as grams of lactic acid per 100 g of product, is equal to

$$\frac{V \times 0,9}{m}$$

where:

V is the volume, in millilitres, of the sodium hydroxide solution used for the titration (9.2.2);

m is the mass, in grams, of the test portion;

0,9 is the conversion factor for lactic acid.

## 11 PRECISION

### 11.1 Repeatability

The absolute difference between the results of two single determinations, carried out simultaneously or in rapid succession by the same operator under the same conditions on identical test material, shall not exceed 0,05 g of lactic acid per 100 g of product.

## 12 TEST REPORT

The test report shall show the method used and the result obtained. It shall also mention any operating details not specified in this International Standard, or regarded as optional, together with details of any incidents likely to have influenced the result.

The test report shall include all information necessary for the complete identification of the sample.

## LITERATURE

Amariglio, S., Contrôle de la qualité des produits laitiers, analyses physiques et chimiques, méthode XIII-4. AFNOR-ITSV (1986).

**REPRESENTATIVE LIST OF LABORATORIES\***

(\*The National Yogurt Association does not endorse any particular laboratories. The following laboratories are, however, believed to be qualified to perform the analyses required for the NYA Seal Program.)

**ABC Research Corporation**

3437 SW 24th Ave  
Gainesville, FL 32607  
Phone: 352 372 0436  
Fax: 352 378 6483  
www.abcr.com

**Ameritech Labs**

12817 20<sup>th</sup> Ave.  
College Point, NY 11356  
Phone: 718.461.0475  
Fax: 718.461.0187

**Analytical Food Laboratories, Inc.**

865 Greenview Dr.  
Grand Prairie, Texas 75050  
Phone: 972 336 0336  
Fax: 972 623 0055  
www.afltexas.com

**Certified Laboratories, Inc.**

200 Express Street  
Plainview, New York 11803  
516-576-1400 phone  
516-576-1410 fax  
800 CERT-LAB  
e-mail: cbesanceney@800certlab.com

6460 Dale Street  
Buena Park, California 90621  
714-562-8622 phone  
714-562-8799 fax  
888 FOOD-LAB

**Medallion Laboratories, Inc.**

9000 Plymouth Ave N  
Minneapolis, MN 55427  
USA  
Phone: 763 764 4453  
Fax: 763 764 4010  
www.MedallionLabs.com

**Minnesota Valley Testing  
Laboratories, Inc.**

Rob True, Sales/Business Development  
PO Box 249, 1126 North Front Street  
New Ulm, MN 56073  
United States  
Phone: 800 782 3557  
Fax: 507 359 2890  
www.mvttl.com

**The National Food Laboratory, Inc.**

6363 Clark Ave  
Dublin, CA 94568  
925-828-1440  
[www.TheNFL.com](http://www.TheNFL.com)

**Silliker Food Science Center**

160 Armory Drive  
South Holland, IL 60473  
Tel. 708/ 225 1435  
Fax. 708/ 225 1536

## National Yogurt Association Seal Program Laboratory Report Form

### A. CULTURE COUNTS AND TITRATABLE ACIDITY

SAMPLE	TOTAL VIABLE CULTURE COUNT – FRESH SAMPLES (CFU/g)	TITRATABLE ACIDITY (for FROZEN yogurt products only)
Beginning of Production Run		
Middle of Production Run		
End of Production Run		

### B. ACTIVITY TEST (at end of code in CFU/g)

Sample A      Before Fermentation: \_\_\_\_\_

After Fermentation: \_\_\_\_\_

Log Increase: \_\_\_\_\_

Sample B      Before Fermentation: \_\_\_\_\_

After Fermentation: \_\_\_\_\_

Log Increase: \_\_\_\_\_

Sample C      Before Fermentation: \_\_\_\_\_

After Fermentation: \_\_\_\_\_

Log Increase: \_\_\_\_\_

PRODUCT: \_\_\_\_\_

MANUFACTURER: \_\_\_\_\_

**CERTIFICATION:** I certify that the information presented in this report is correct and has been completed by my laboratory, which is independent of the company applying for the NYA Seal.

Laboratory

Name/Address: \_\_\_\_\_

Lab Manager Signature: \_\_\_\_\_

Date: \_\_\_\_\_

**National Yogurt Association  
Live and Active Cultures Seal Application**

*A separate application must be completed for each product. Each application must be accompanied by a nonrefundable fee of \$2,500 per product line payable to the National Yogurt Association.*

Company: \_\_\_\_\_

Address: \_\_\_\_\_ Phone: \_\_\_\_\_

\_\_\_\_\_ Fax: \_\_\_\_\_

\_\_\_\_\_ Email: \_\_\_\_\_

Are a producer and/or distributor of yogurt in the United States? Yes \_\_\_\_ No \_\_\_\_

Product: \_\_\_\_\_

Shelf life of product: \_\_\_\_\_

List other brands name(s) of product, if marketed under more than one name: \_\_\_\_\_

Were the required analytical tests conducted in accordance with the protocols set forth in Appendix A of the NYA Seal Program Procedures? \_\_\_\_\_ (Please attach test results.)

Were the analytical tests conducted by a state or USDA-certified independent laboratory? \_\_\_\_\_

**Laboratory Contact Information:**

Name/Contact: \_\_\_\_\_

Address: \_\_\_\_\_

*All applications, attachments, test results, record of any action by the Seal Program Staff, renewal forms, etc. will be provided to any member of the public upon written request.*

*If NYA approves the application, the company ("the licensee") agrees to hold NYA ("the licensor") harmless; and to defend at licensee's expense, all actions arising out of the licensee's use of the NYA Seal on a product that does not contain the levels of live and active cultures specified by licensor for use of the seal, provided that licensee fraudulently or negligently misrepresented the levels of live and active cultures in the product identified in this application or otherwise misrepresented any material fact. The licensee shall indemnify the licensor against all judgments, fines, amounts paid in settlement, and reasonable expenses including attorney's fees, as actually and necessarily incurred by licensor in connection with such action, suit, investigation or proceeding or in connection with any appeal therein.*

*By signing this application, you certify that the product was tested by the above named laboratory and that the results of the test were in compliance with the guidelines set forth in Appendix A of the NYA Seal Program Procedure.*

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Name: \_\_\_\_\_ Title: \_\_\_\_\_