Designation: D7817 – 12 (Reapproved 2016)

Standard Test Method for Enumeration of Yeast and Mold in Raceway Brine, Brine-Cured Hides and Skins[1]

This standard is issued under the fixed designation D7817; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the enumeration of yeast and mold. This test method is applicable to raceway brine, brine-cured hides and skins, and pre-charge raceway liquor.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D6715 Practice for Sampling and Preparation of Fresh or Salt-Preserved (Cured) Hides and Skins for Chemical and Physical Tests

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

3. Summary of Test Method

3.1 Samples of brine-cured hides and skins, raceway brine, or pre-charge raceway liquor are serially diluted and plated on agar containing 7 % NaCl and an antibiotic solution. The plates are incubated at 20 – 25°C for 5 days.

4. Significance and Use

4.1 This test method enumerates salt tolerant yeast and mold, and under the conditions of this test method those are equated as halophilic organisms. Salt tolerant yeast and mold have been known to cause damage to hides and skins in raceway brine.

5. Apparatus

5.1 *Incubator,* 20 – 25°C.

5.2 *Colony counter*—(not mandatory, but highly recommended).

5.3 *Sterile pipets.*

5.4 *Stomacher,* for mixing initial dilution. (If stomacher is unavailable, hand-mix.)

5.5 *Balance.*

5.6 *Sterile petri dishes.*

5.7 *Autoclave (sterilizer)*—(Check the effectiveness of sterilization weekly. For example, place spore suspensions or strips of *Bacillus stearothermophilus* (commercially available) inside glassware for a full autoclave cycle. Follow manufacturer's directions for sterilization of specific media.)

5.8 *pH meter.*

5.9 *Waterbath,* 45 ± 1°C.

5.10 *Stomacher bags,* or sterile, sealable quart plastic bag (e.g. food storage type, sterile bag).

5.11 *Cutting tool,* sterile (e.g. scalpel blade and forcep, as needed for cutting cured hides and skins).

5.12 *Vortex mixer,* for mixing dilution tubes (optional).

5.13 *Autoclave thermometer.*

6. Reagents and Materials

6.1 *Butterfield's Phosphate Stock Solution*—Dissolve 34 g KH_2PO_4 (Potassium Phosphate monobasic) in 500 mL DI water. Adjust the pH to 7.2 ± 0.1 with 1N – 6N NaOH. Bring volume to 1 L with DI water. Sterilize for 15 min at 121°C.

NOTE 1—Typical autoclave setting is 120 – 124°C. (See 5.7.)

6.2 *Butterfield's Phosphate Diluent with salt (BPD w/salt)*—Take 1.25 mL of Butterfield's Phosphate Stock solution (6.1) and bring to 1 L with DI water, then add 77 g of salt (NaCl) per litre prior to autoclaving. Dispense into 1-L bottles and 9-mL dilution tubes. Sterilize for 15 min at 121°C. (See Note 1.)

[1] This test method is under the jurisdiction of ASTM Committee D31 on Leather and is the direct responsibility of Subcommittee D31.02 on Wet Blue.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

6.3 *Potato Dextrose Agar (PDA).*

6.4 *Antibiotic solution*—(Chloramphenicol)[3] – (needed to inhibit bacterial growth on agar).

6.5 *Distilled or deionized water.*

6.6 *Salt (NaCl),* Sodium chloride – reagent grade.

6.7 *1N – 6N NaOH.*

6.8 *Bacillus stearothermophilus* spore suspensions or strips (commercially available), or equivalent.

7. Hazards

7.1 All reagents and chemicals should be handled with care. Before using any chemical, read and follow all safety precautions and instructions on the manufacturer's label or MSDS (Material Safety Data Sheet).

8. Sampling

8.1 The specimen shall be sampled in accordance with Practice D6715, and placed in sterile containers.

9. Preparation of Potato Dextrose Agar and Antibiotic Solution

9.1 Prepare the antibiotic stock (10 000 ppm) solution by dissolving 1 g of chloramphenicol in 100 mL sterile deionized or distilled water. Store this stock solution in a dark location at ≤5°C for up to two months.

9.2 Suspend 39 g of Potato Dextrose Agar in 1 L of deionized or distilled water and heat to boiling to dissolve completely.

9.3 Add 77 g of NaCl per litre of agar. Add 10 mL of chloramphenicol stock solution per litre of agar to give a concentration of 100 ppm. Sterilize in the autoclave for 15 min at 121°C. (See Note 1.) Cool to 45 ± 1°C in a waterbath. Once medium has been tempered, it can be held for 2 – 3 h before use, provided the water level in the waterbath is 2 – 3 cm above the surface of the agar. Final pH of the agar: 5.6 ± 0.2.

10. Procedure

10.1 Using a sterile scalpel, aseptically weigh a 20 ± 0.1 g specimen in a sterile bag. For brine-cured hides and skins, include both flesh and hair side.

10.2 Add 180 g of BPD w/salt (6.2) diluent into the same sterile bag (10.1). Stomach or hand-massage for 1 min. This provides a 1:10 dilution.

10.3 Prepare the following sample dilutions: 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} (see Fig. 1).

10.3.1 *Control Blank*—In 10.5, pour melted media that has been previously tempered to 45 ± 1°C into a dish, then continue with 10.6 as with the sample plates.

Example: To obtain a 10^{-2} dilution, mix the 10^{-1} dilution and pipet 1 mL of that 10^{-1} dilution into a 9-mL dilution tube.

NOTE 2—When transferring the aliquots between the tubes, the analyst must use a different pipet or pipet tip for each transfer.

10.4 Pipet 1 mL of each dilution into the appropriate, separate petri dishes.

10.5 Pour prepared agar (9.3) that has been previously tempered to 45 ± 1°C into the dish.

[3] The sole source of supply known to the committee at this time is Sigma-Aldrich, Cat. # C0378 (25 g). If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

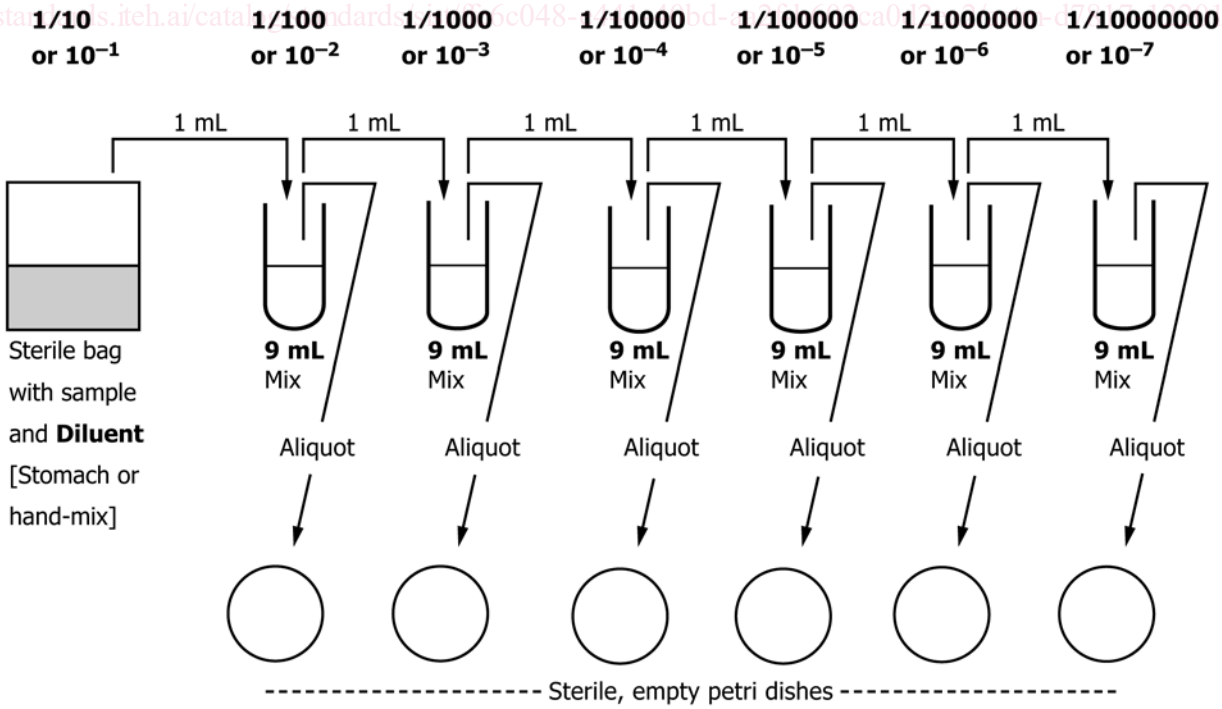

FIG. 1 Plating