



Standard Test Method for Enumeration of Halophilic and Proteolytic Bacteria in Raceway Brine, Brine-Cured Hides and Skins¹

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1. Scope

1.1 This test method covers the enumeration of bacteria that can tolerate high salt concentrations or can hydrolyze protein/collagen, or both. 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

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

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

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 casein from skim milk. The plates are incubated under aerobic conditions at 35°C for 48 h. After incubation, to determine salt tolerant (halophilic) bacteria, all colonies are counted. To determine bacteria that

can hydrolyze protein (proteolytic), the plates are flooded with dilute acid and the colonies showing a “halo” (semi-clear zones) are counted.

4. Significance and Use

4.1 This test method enumerates salt tolerant (halophilic) bacteria, and proteolytic bacteria that are also salt tolerant. Under the conditions of this test method those bacteria are equated as halophilic organisms. Salt tolerant proteolytic bacteria have been known to cause damage to hides and skins in raceway brine.

5. Apparatus

5.1 *Incubator*, 35 ± 1°C.

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

5.3 *Sterile pipets*.

5.4 *Bent glass rods* (“hockey-stick”), sterile. (If non-sterile, will need to flame sterilize.)

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

5.6 *Balance*.

5.7 *Sterile petri dishes*.

5.8 *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.)

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

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

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

5.12 *pH meter*.

5.13 *Waterbath*, 45 ± 1°C.

5.14 *Autoclave thermometer*, or equivalent for monitoring autoclave temperature.

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.