13 Microbiological Methods

Coagulase-positive Staphylococcus

(American Association of Cereal Chemists – AACC-Method 42-30B)
First approval 12-15-72; revised 10-30-75; reviewed 10-28-81

1 Scope

This Standard specifies a method for the enumeration of microorganisms by means of the colony count techniques at 35-37°C. The method is applicable to all cereal and other food products.

2 Apparatus

2.1 Sterile sampling devices suitable for product; scoop, trier, etc.

2.2 Sterile sample jars or containers with suitable covers or sterile whirl-pak plastic bags.

2.3 Sterile glassware or sterile disposable petri dishes, transfer pipettes, and culture tubes.

2.4 Incubator adjusted to 35-37°C.

2.5 Autoclave for sterilising app and media.

2.6 Balance; sensitivity 30 mg with wts.

3 Reagents

3.1 *Trypticase soy broth, with 10.0% NaCl:* Add 95 g NaCl to 1 L of soln of 17.0 g Trypticase or Tryptose (pancreatic digest of casein), 3.0 g Phytone (papaic digest of soy meal), 5.0 g NaCl, 2.5 g K_2HPO_4, and 2.5 g glucose. Heat gently if necessary. Dispense into 16-20 mm diam culture tubes to depth of 5-8 cm. Autoclave 15 min at 120°C. Final pH, 7.3 ± 0.1.

3.2 Baird-Parker (egg tellurite glycine pyruvate agar, ETGPA):

a. *Base I medium:* Suspend 10.0 g Tryptone, 5.0 g beef ext, l.0 g yeast ext, 10 g Na pyruvate, 12.0 g glycine, 5.0 g LiCl $6H_20$, and 20.0 g agar in 950 ml water. Heat to boiling with frequent agitation to dissolve ingredients completely. Dispense 95 ml portions into screw-capped bottles. Autoclave 15 min at 121°C. Final pH,7.0 ± 0.2 at 25°C. Store ≤ 1 mo at 4 ± 1°C.

Enrichment — Bacto egg yolk tellurite enrichment (Difco Laboratories), or prep as follows: Soak fresh eggs ca 1ÿmin in diln of saturated $HgCl_2$ soln (1 + 1000). Aseptically crack eggs and separate yolks from whites. Blend yolk and physiological saline soln in high-speed blender 5 sec. Physiological saline soln: dissolve 8.5 g NaCl in 1 L water. Autoclave 15 min at 121°C and cool to room temp. To 50 ml egg yolk emulsion add 10 ml filter sterilised 1% K tellurite soln. Mix and store at 4 ± 1°C.

b. *Complete medium:* Add 5 ml warmed enrichment to 95 ml molten agar medium cooled to 45-50°C. Mix well, avoid bubbles, and pour 15-18 ml into sterile 100 x 15 mm petri dishes. Store plates 48 hr at 4 ± 1°C before use. Medium should be densely opaque; do not use non-opaque plates. Dry plates before use by one of the following methods: 1) in convection oven or incubator 30 min at 50°C with lids removed and agar surface downward; 2) in forced draft oven or incubator 2 hr at 50°C with lids on and agar surface upward; 3) in incubator 4 hr at 35°C with lids on and agar surface upward; or 4) on laboratory bench 16-18 hr at room temp with lids on and agar surface upward .

3.3 *Desiccated coagulase plasma (rabbit) with EDTA*: Reconstitute according to manufacturer's directions. If not available, reconstitute desiccated coagulase plasma (rabbit) and add Na_2H_2EDTA to final concn of 0.1 % reconstituted plasma.

3.4 *Diln blanks:* Peptone or Butterfield's phosphate buffered diluent. Use 6 oz bottles of resistant glass closed with rubber stoppers or screw caps. Use distd water. Prep 0.1% peptone soln by adding 1 g peptone (Difco) to each 1000 ml distd water. Adjust pH to 6.8. Measure soln accurately into bottles so they contain 99 ml after sterilisation. Autoclave 15 min at 121°C (15 lb pressure). Prep Butterfield's phosphate buffer (stock) by dissolving 34 g KH_2PO_4 in 500 ml water adjusting pH to 7.2 with ca 175 ml 1 N NaOH, and dilg to 1000 ml. Store in refrigerator. Dil 1.25 ml stock soln to 1000 ml with distd water; prep diln blanks with this soln so they contain 99 ml after sterilisation. Autoclave 15 min at 121°C (15 lb pressure).

3.5 *Brain heart infusion (BHI) broth:* To rehydrate BHI broth, dissolve 3.7 g media in 100 ml distd water and sterilise in autoclave 15 min at 121°C (15 lb pressure).

3.6 *Trypticase soy agar:* Suspend 4 g powder in 100 ml distd water. Let stand 6 min and mix thoroughly. Heat gently with occasional agitation 1 min or until soln is complete. Autoclave 15 min at 121°C. Let cool and re-autoclave 15 min at 121°C.

3.7 *Tris buffer:* Dissolve 6.05 g tris (hydroxymethyl)aminomethane and 1 ml 0.11% $CaCl_2$ in 500 ml distd water, adjust pH to 9.0 with 1 N HCl, and make to 1000 ml with distd water.

3.8 *Toluidine blue-DNA agar (TDA plates):* Dissolve 0.3 g Bacto-DNA, 10 g Bacto-Agar (both from Difco), and 10 g NaCl in 1000 ml tris buffer. Steam for 30-40 min. Cool melted agar to 45°C and add 3 ml 3% toluidine blue soln in distd water.

Mix and pipette 5 ml into plastic petri dishes (15 x 60 mm) with lids open to allow steam to escape. After agar solidifies store plates at 5.

4 Procedure

4.1 Use sample taken and prepared as directed in "Microbiological examination"

Note: Steps in the original method describing the preparation of sample for microbiological examination are deleted.

4.2 With food samples that are normally expected to contain ≤100 Staphylococcus aureus/ g, inoculate three tubes of Trypticase soy broth (TSB) with 10.0% NaCl at each test diln with l ml aliquots of decimal dilns of sample. Maximum dilns of sample must be high enough to yield negative end point. With food samples expected to contain ≥100 S. aureus/g, inoculate food slurry and two addnl decimal dilns onto BPA plates. Inoculate 0.5 ml of each diln onto each of two BPA plates. Spread inoculum over entire surface of BPA plates with sterile bent glass rods. Incubate TSB tubes and/or BPA 48 hr at 35-37°C.

4.3 Using 3 mm loop, transfer 1 loop full from each growth positive TSB tube to dried BPA plates; streak so as to obtain isolated colonies. Incubate 45-48 hr at 35-37°C. Count colonies on BPA (from foods directly plated) as groups with characteristics listed in 4.4 and proceed through step 4.5.

4.4 From each plate showing growth, pick one colony of each of the following types that are convex, shiny black, and (a) with or without narrow, grey-white margin, surrounded by clear zone extending into opaque medium; (b) with or without narrow, grey-white margin, surrounded by clear zone with inner opaque zone; or (c) with or without grey-white margin, 1.0-1.5 mm diam. Transfer colonies to agar slants contg any suitable maintenance medium. Incubate slants (Trypticase soy agar) at 35-37°C until growth is visible. Remove small amt of growth and emulsify in drop of 3% H_2O_2 on microscope slide. Discard cultures that do not produce immediate and vigorous bubbling.

4.5 Inoculate tubes contg 0.5 ml BHI broth with small amt of growth from maintenance slant. Do not transfer large clumps of growth. Incubate 18-24 hr at 35-37°C

Tube coagulase lest: Add 0.2 ml BHI culture to 0.5 ml reconstituted coagulase plasma-EDTA, mix thoroughly, and incubate at 35-37°C for 6 hr. Observe for clotting of plasma at hourly intervals without disturbing clot (gently tilting or slanting). Test positive and negative controls with known cultures.

Optional: Heatstable nuclease (DNAse) assay: Boil balance of BHI culture (about 0.3 ml) for 15 min and cool. Cut 2 mm diam wells in TDA plates with 13-gauge cannula. Fill duplicate wells with boiled BHI culture and incubate 4 hr at 35-37°C. Appearance of pink zone around well is positive indication of DNAse. Test same positive and negative cultures used in tube coagulase test as controls in TDA plates. Cultures positive for clot formation in coagulase test and also positive for pink zone (in TDA) for DNAse are coagulase-positive Staphylococcus or S. aureus. Report coagulase-positive staphylococci per ml or g from BPA plates (plated directly with food slurry and its dilns) by multiplying no. of coagulase-positive colonies from the three groups with appropriate diln factor. In case of food enriched in TSB for Staphylococcus, report coagulase-positive staphylococci most probable number(MPN/g from Tables of MPN values).

Optional: Test for enterotoxigenicity: Some strains of *S. aureus* may produce enterotoxins. *Test S. aureus* cultures for production of enterotoxin serologically by method outlined in latest edition of Bacteriological Analytical Manual of the FDA, HEW, Washington, DC.

5 References

1. Association of Official Analytical Chemist. 1980. Official Methods of Analysis, 13th ed. Secs. 46.017, p. 826; 46.075-46.076, p. 841.

2. Difco Laboratories, Inc. Difco Manual, 9th ed. Detroit, MI.

3. U.S. Food and Drug Administration. 1978. Bacteriological Analytical Manual for Foods, chapt. XI. Washington, DC.