AOAC Official Method 966.23
Microbiological Methods

First Action 1966
Final Action 1989

(For the determination of aerobic plate count, most probable number of coliform bacteria, and *Escherichia coli* and *Staphylococcus* in products such as frozen cooked meat, poultry, and vegetable products; cooked and/or breaded seafood; bakery products; salads; tree nut meats; and ingredients of food laboratory samples collected during sanitation inspections of food-producing establishments, unless specific directions are given for that product.)

A. Media and Reagents

Ingredients and reagents used to prepare the following media may be products of any manufacturer if comparative tests show that satisfactory results are obtained. Use pure carbohydrates suitable for biological use, ACS reagent grade inorganic chemicals, and dyes certified by the Biological Stain Commission for use in media.

For convenience, dehydrated media of any brand equivalent to formulation may be used. Test each lot of medium for sterility and growth-promoting qualities of suitable organisms (e.g., inoculate media containing lactose with coliform bacteria, *Staphylococcus* media with *Staphylococcus,* etc.).

Determine pH before autoclaving with pH meter standardized against standard buffers, **964.24** (*see* A.1.04). Adjust pH, when necessary, by adding 1M NaOH or 1M HCl so that stated final pH results after autoclaving.

Use sterile glass or plastic, 100 ×15 mm, Petri dishes.

(**a**) *Plate count agar.*—*See* **940.36A**(**g**) (*see* 17.1.02).

(**b**) *Lauryl sulfate tryptose broth.*—Dissolve 20.0 g Trypticase or tryptose (pancreatic digest of casein), 5.0 g NaCl, 5.0 g lactose, 2.75 g K_2HPO_4, 2.75 g KH_2PO_4, and 0.1 g sodium lauryl sulfate in 1 L H_2O with gentle heat, if necessary. Dispense 10 mL portions into 20 ×150 mm test tubes containing inverted 10 ×75 mm fermentation tubes. Autoclave 15 min at 121°C. Final pH, 6.8 ± 0.1.

(**c**) *Brilliant green lactose bile* (*BGLB*) *broth.*—Dissolve 10.0 g peptone and 10.0 g lactose in ca 500 mL H_2O. Add solution (pH 7.0–7.5) of 20 g dehydrated oxgall or oxbile in 200 mL H_2O. Dilute to 975 mL and adjust pH to 7.4. Add 13.3 mL 0.1% solution of brilliant green, and dilute to 1 L with H_2O. Filter through cotton and dispense 10 mL portions into 20 ×150 mm test tubes containing inverted 10 ×75 mm fermentation tubes. Autoclave 15 min at 121°C. Final pH, 7.2 ± 0.1.

(**d**) *Eosin methylene blue agar* (*Levine*).—*See* **940.36A**(**d**) (*see* 17.1.02).

(**e**) *Baird-Parker medium* (*egg tellurite glycine pyruvate agar, ETGPA*).—(*1*) *Basal medium*.—Suspend 10.0 g tryptone, 5.0 g beef extract, 1.0 g yeast extract, 10 g sodium pyruvate, 12.0 g glycine, 5.0 g $LiCl·6H_2O$, and 20.0 g agar in 950 mL H_2O. Heat to bp 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 month at 4 ± 1°C.

(*2*) *Enrichment.*—Bacto EY tellurite enrichment (Difco Laboratories or equivalent) or prepare as follows: Soak fresh eggs ca 1 min in dilution of saturated $HgCl_2$ solution (1 + 1000, w/v). Aseptically crack eggs and separate yolks from whites. Blend yolk and physiological saline solution, **940.36B**(**c**) (*see* 17.1.02), (3 + 7, v/v) in high-speed blender ca 5 s. To 50 mL egg yolk emulsion, add 10 mL filter-sterilized 1% potassium tellurite solution (w/v). Mix and store at 4 ± 1°C.

(*3*) *Complete medium.*—Add 5 mL warmed enrichment to 95 mL molten basal medium cooled to 45–50°C. Mix well, avoiding bubbles, and pour 15–18 mL into sterile 100 ×15 mm Petri dishes. Store plates at room temperature (≤25°C) for ≤5 days before use. Medium should be densely opaque; do not use nonopaque plates. Dry plates before use by one of the following methods: (*a*) in convection oven or incubator 30 min at 50°C with lids removed and agar surface downward; (*b*) in forced-draft oven or incubator 2 h at 50°C with lids on and agar surface upward; (*c*) in incubator 4 h at 35°C with lids on and agar surface upward; or (*d*) on laboratory bench 16–18 h at room temperature with lids on and agar surface upward.

(*4*) *Interpretation.*—Colonies of *S. aureus* are typically circular, smooth, convex, moist, 2–3 mm in diameter on uncrowded plates, gray-black to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone (precipitate) and frequently with outer clear zone; colonies have buttery to gummy consistency when touched with inoculating needle. Occasional nonlipolytic strains may be encountered which have same appearance, except that surrounding opaque and clear zones are absent. Colonies isolated from frozen or desiccated foods which have been stored for extended periods are frequently less black than typical colonies and may have rough appearance and dry texture.

(**f**) *Trypticase* (*tryptic*) *soy broth with 10% sodium chloride.*—Add 95 g NaCl to 1 L of solution of 17.0 g Trypticase or tryptose (pancreatic digest of casein), 3.0 g Phytone (papaic digest of soya 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 diameter tubes to depth of 5–8 cm. Autoclave 15 min at 121°C. Final pH, 7.3 ± 0.2.

(**g**) *EC broth.*—Dissolve 20.0 g Trypticase or tryptose (pancreatic digest of casein), 1.5 g Bacto bile salt No. 3 or bile salt mixture, 5.0 g lactose, 4.0 g K_2HPO_4, 1.5 g KH_2PO_4, and 5.0 g NaCl in 1 L H_2O. Dispense 8 mL into 16 ×150 mm test tubes containing inverted 10 ×75 mm fermentation tube. Autoclave 15 min at 121°C. Final pH, 6.9 ± 0.1.

(**h**) *Brain-heart infusion.*—*See* **967.25A**(**r**) (*see* 17.9.01). Dispense into bottles or tubes for storage and autoclave 15 min at 121°C.

(**i**) *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 concentration of 0.1% in reconstituted plasma.

(**j**) *Tryptophane broth.*—*See* **940.36A**(**h**) (*see* 17.1.02) but dispense in 10 mL portions.

(**k**) *Buffered glucose broth (MR-VP medium).*—*See* **940.36A**(**b**) (*see* 17.1.02).

(**l**) *Koser's citrate broth.*—*See* **940.36A**(**e**) (*see* 17.1.02).

(**m**) *Butterfield's buffered phosphate diluent.*—(*1*) *Stock solution.*—Dissolve 34.0 g KH_2PO_4 in 500 mL H_2O, adjust to pH 7.2 with ca 175 mL 1M NaOH, and dilute to 1 L. Store in refrigerator. (*2*) *Diluent.*—Dilute 1.25 mL stock solution to 1 L with H_2O. Prepare dilution blanks with this solution, dispensing enough to allow for losses during autoclaving. Autoclave 15 min at 121°C.

B. Preparation of Test Sample

(Prepare all decimal dilutions with 90 mL sterile diluent plus 10 mL previous dilution unless otherwise specified. Shake all dilutions 25 times in 30 cm arc. Pipets must accurately deliver required volume. Do not use to deliver <10% of their total volume. For example, to deliver 1 mL, do not use pipet >10 mL; to deliver 0.1 mL, do not use pipet >1 mL.)

(**a**) *Frozen and/or prepared foods.*—Use balance with capacity of ≥2 kg and sensitivity of 0.1 g to aseptically weigh 50 g unthawed (if frozen) test sample into sterile high-speed blender jar. Add 450 mL diluent, (**m**)(*2*), and blend 2 min. (If necessary to temper frozen test sample to remove 50 g portion, hold ≤18 h at 2–5°C.) Not >15 min should elapse from time test sample is blended until all dilutions are in appropriate media.

If entire test sample consists of <50 g, weigh portion equivalent to ½ test sample and add volume of sterile diluent required to make 1:10 dilution. Total volume in blender jar must completely cover blades.

(**b**) *Tree nut meat halves and larger pieces.*—Aseptically weigh 50 g test sample into sterile jar. Add 50 mL diluent, (**m**)(*2*), and shake vigorously (50 times through 30 cm arc) to obtain 10^0 dilution. Let stand 3–5 min and shake just before making serial dilutions and inoculations.

(**c**) *Nut meal.*—Aseptically weigh 10 g test sample into sterile jar. Add 90 mL diluent, (**m**)(*2*), and shake vigorously (50 times through 30 cm arc) to obtain 10^{-1} dilution. Let stand 3–5 min and shake to resuspend just before making serial dilutions and inoculations.

C. Aerobic Plate Count

Seed duplicate Petri dishes in dilutions of 1:10, 1:100, 1:1000, etc., using plate count agar, (**a**). Ordinarily, 1:100 through 1:10 000 are satisfactory. Place 1 mL appropriate dilution in each plate, and add molten agar (cooled to 42–45°C) within 15 min from time of original dilution. Incubate 48 ± 2 h at 35°C and count duplicate plates in suitable range (30–300 colonies). If plates do not contain 30–300 colonies, record dilution counted and note number of colonies found. Average counts obtained and report as aerobic plate count/g.