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Bacteriological Analytical Manual

Chapter 23: Methods for Cosmetics

July 2024 Edition

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Revision History

- July 2024: Sections H.3 and I updated.
- April 2024: Sections A, F and G updated to include protocol to analyze wet wipes including stomaching and vortexing options.
- December 2021: Revision to Section J.
- July 2021: Revision removes Figure 1, archives biochemical tests to confirm Pseudomonas aeruginosa, add new contact for fungal isolates, and indexing and minor wording edits for clarity.
- The original version of this Chapter is available as an archived method.
- July 2017: Revision to Section H-1 and H-2.
- January 2017: Section H-1 and H-2. Additional 1 ml of 10-1 dilution is analyzed.
- May 2016: Section H-1. Changed dilution range from 10-1 - 10-6 to 10-1 - 10-3.
- May 2016: Section H-4. Deleted the entire section: Screening test for total numbers of microorganisms.
- May 2016: Section: Identification of Microbes updated: A.1. Gram-positive rods. Identify Bacillus-like rods if isolated from aerobic plates.
- August 2001: Section: Identification of Microbes. Revised Part D and added reference 2b.
- August 2001: M79 formulation corrected.

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Introduction

The ability of microorganisms to grow and reproduce in cosmetic products has been known for many years. Microorganisms may cause spoilage or chemical changes in cosmetic products and injury to the user (4,5,10,14-16,20,21). Methods for isolation of microorganisms from cosmetic products are direct colony counts and enrichment culturing. Products that are not soluble in water are initially treated to render them miscible before isolation procedures are conducted. Dilution and plating media that partially inactivate preservative systems commonly found in cosmetic products are used. The isolated microorganisms are identified by routine microbiological methods or by commercial identification kits.

A. Equipment and materials

1. Pipets, sterile, 1, 5, and 10 ml, graduated
2. Gauze pads, sterile, 4 × 4 inch
3. Sterile instruments: forceps, scissors, scalpel and blades, spatulas, and microspatulas
4. Test tubes, screw-cap, 13 × 100, 16 × 125, and 20 × 150 mm
5. Dilution bottles, screw-cap
6. Balance, sensitivity of 0.01 g
7. Petri dishes, sterile, plastic, 15 × 100 mm
8. Bent glass rods, sterile
9. Incubators, 30 ± 2°C and 35 ± 2°C
10. Anaerobic atmosphere generating envelopes, indicator strips, and jars (BBL, Oxoid or equivalent), or anaerobic incubator, 35 ± 2°C, or anaerobic glove box, 35 ± 2°C.
11. Candle jars or CO_2 incubator, 35 ± 2°C.
12. Laminar flow hood with HEPA filter, if available
13. Vitek or equivalent automated computerized identification system
14. Homogenizer, such as stomacher
15. Sterile bags for homogenizer (with filter preferred)
16. Sterile large volume screw-cap conical tubes

B. Media for enumeration and identification of Gram-positive bacteria and fungi

1. Anaerobe agar (M11)
2. Bile esculin agar (M18)
3. Brain heart infusion (BHI) agar and broth (M24)
4. Malt extract agar (MEA) (M93)
5. Potato dextrose agar (PDA) (M127)
6. Mannitol salt agar (M97)
7. Modified letheen agar (MLA) (M78) and broth (MLB) (M79)
8. Oxidative-fermentative (OF) test medium (M117)
9. Sabouraud's dextrose broth (M133)
10. Blood agar base (M20a)
11. Starch agar (M143)
12. Trypticase (tryptic) soy agar (TSA) (M152) and broth (TSB) (M154)
13. Baird-Parker (BP) agar (M17)
14. Catalase test (R12)
15. Vogel-Johnson (VJ) agar (optional) (M176)
16. Commercial bacterial identification kit (API or equivalent)

C. Media for identification of Enterobacteriaceae

1. Andrade's carbohydrate broth and indicator (M13) for testing metabolism of rhamnose, mannitol, sorbitol, arabinose
2. Lysine iron agar (M89)
3. Malonate broth (M92) or phenylalanine malonate broth (Difco)
4. Motility-indole ornithine medium (M99)
5. MR-VP broth (M104)
6. Simmons citrate agar (M138)
7. Triple sugar iron (TSI) agar (M149)
8. Christensen's urea agar (M40)
9. MacConkey agar (M91)
10. Lysine decarboxylase medium for Gram-negative nonfermentative bacteria (M88)
11. Phenylalanine deaminase agar (M123) **(see also C-3, above)**
12. API 20E, Roche Enterotube, or other equivalent identification kits
    Incubate all biochemical tests using media in B and C, above, at 35-37°C for 18-24 h, except malonate broth (48 h) and MR-VP broth (48 h or longer).

D. Media and reagents for identification of Gram-negative nonfermentative (NF) bacilli

1. Acetamide medium (M2)
2. Clark's flagellar stain (R14)
3. Esculin agar, modified (CDC) (M53)
4. Nutrient gelatin (CDC) (M115)
5. Indole medium (M64) and indole medium (CDC) (M65)
6. King's B medium (M69)
7. Lysine decarboxylase (LDC) medium for Gram-negative NF bacteria (M88)
8. Motility nitrate medium (M101)
9. Nitrate broth, enriched (CDC) (M109)
10. King's OF basal medium (M70) for testing metabolism of sucrose, lactose, fructose, esculin, xylose, glucose (dextrose), mannitol, salicin, sorbitol, and maltose
11. Oxidase test strips
12. Christensen's urea agar (M40)
13. Decarboxylase basal medium (for arginine decarboxylase) (M44)
14. Yeast extract (YE) agar (M181)
15. Pseudomonas agars F (M128 and P (M129) (Difco)
16. Cetrimide agar (Pseudosel™, BBL; Difco), or equivalent (M37)
17. Glycerol, sterile (Difco), or equivalent
18. API, NFT, or other equivalent commercial identification system
19. Koser's citrate broth (M72)

E. Other media and reagents

1. Aqueous solution of 70% ethanol and 1% HCl (v/v) **or** 4% iodine in 70% ethanol solution or 2% glutaraldehyde solution
2. Tween 80 (Polysorb 80)
3. Ethanol, 95% (v/v)
4. Lyophilized rabbit coagulase plasma with EDTA
5. 3% (v/v) Aqueous solution of hydrogen peroxide
6. Gram stain (R32) and endospore stain (R32a)
7. Cooked meat medium (M42)

F. Handling of cosmetic samples for microbiological analysis

Analyze samples as soon as possible after their arrival. If necessary, store samples at room temperature. Do not incubate, refrigerate, or freeze samples before or after analysis. Inspect samples carefully before opening and note any irregularities of sample container. Disinfect surface of sample container with aqueous mixture of 70% ethanol (v/v) and 1% HCl (v/v) or other disinfectant (**see** E-I) before opening and removing contents. Use laminar flow hood if possible. Dry surface with sterile gauze before opening. Use representative portion of contents for microbial analysis.

For products weighing less than 1 g (ml), analyze entire contents. If only one sample unit is available and multiple analyses are requested (i.e., microbial, toxicological, and chemical), take subsample for microbiological examination before those for other analyses. In this situation, amount of subsample used for microbial analysis will depend on other analyses to be performed. For example, if total sample content is 5 ml, use 1 or 2 ml portion for microbial analyses.

G. Preliminary sample preparation

Amounts of sample and diluent given here can be adjusted according to amount of sample available. If sample has many subsamples, amount of test material can be increased and workload streamlined by compositing. Analysts should use their best judgment as to when and how much material to composite.

1. **Liquids**. Decimally dilute 1 ml liquid directly into 9 ml modified letheen broth (MLB) in 20 × 150 mm screw-cap test tube for the 10^{-1} dilution.
2. **Solids and powders**. Aseptically remove and weigh 1 g sample into 20 × 150 mm screw-cap test tube containing 1 ml sterile Tween 80. Disperse product in Tween 80 with sterile spatula. Add 8 ml sterile MLB and mix thoroughly. This will be the 10^{-1} dilution.
3. **Cream and oil-based products**. Aseptically remove and weigh 1 g sample into 20 × 150 mm screw-cap tube containing 1 ml sterile Tween 80 plus five to seven 5-mm glass beads (or ten to fifteen 3-mm glass beads). Mix total contents with Vortex mixer. Adjust total volume to 10 ml with sterile MLB (8 ml) for the 10^{-1} dilution.
4. **Aerosols of powders, soaps, liquids, and other materials**. Decontaminate nozzle of spray can as much as possible by swabbing with gauze pad moistened with 70% (v/v) aqueous ethanol. Expel some product to flush out nozzle; then spray appropriate amount into tared dilution bottle, e.g., 1 g of product into 9 ml sterile MLB. Thoroughly mix product and broth, and reweigh. This will be a 10^{-1} dilution if exactly 1 g of sample was obtained.
5. **Anhydrous materials**. Treat as in G-2 or G-3, as appropriate.
6. **Wet wipes.** Aseptically remove one sheet of wipe from a retail package (a subsample). The location of the sampled sheet in the wipe container is described in the Note below. Tare a sterile bag, then place the wipe sheet into the bag and weigh. Into the bag, add an equal amount of sterile Tween 80 and an 8-fold amount of sterile MLB to make a 10^{-1} dilution. For example, for 5 g of a wipe, add 5 mL of Tween 80 and 40 mL of MLB. Place

the bag in a stomacher and mix the contents by stomaching at 230 rpm for 1 min. Alternatively, tare a sterile conical tube, then place the wipe sheet into the conical tube and weigh. Into the tube, add ten 4-mm glass beads (or five to seven 5-mm glass beads or ten to fifteen 3-mm glass beads), an equal amount of sterile Tween 80, and an 8-fold amount of sterile MLB to make a 10^{-1} dilution. Mix the contents by a vortex at maximum speed for 30 seconds.
Note: The amount and types of microorganisms present in wipes may vary at different locations of a container (a subsample). Therefore, it is recommended to take a wipe sheet from different locations when analyzing multiple subsamples. For example, in a stack of wipes, for sub #1 and #2, the one sheet is taken from the top one-third section of the stack; for sub #3 and #4, the one sheet is taken from the middle one-third section; for sub #5, the one sheet is taken from the bottom one-third section.

H. Microbiological enumeration and growth

1. Aerobic plate count (APC)

Use spread plate technique to facilitate recognition of different colony types. Decimally dilute the cosmetic preparation (see G, above) in MLB to obtain a complete dilution series from 10^{-1} to 10^{-3}. Use a new sterile pipet to transfer 1.0 ml of the current dilution into 9 ml of fresh MLB to make the next decimal dilution.

Mix the dilutions thoroughly and **perform all plating in duplicate**. Label petri dishes containing modified letheen agar (MLA) accordingly.

For each dilution (10^{-1} to 10^{-3} dilution), spread 0.1 ml onto MLA. The dilution factors are 100, 1000, and 10,000, respectively.

In addition, plate 1 ml of 10^{-1} dilution onto one set of either two (0.5 ml each), or three (0.3, 0.3, 0.4 ml each) MLA plates. Regardless of the number of plates used in plating, the total volume should add up to 1 ml. Do the same for a duplicate set. The dilution factor is 10.

Conduct plating in the same manner for fungi count in H-2, and anaerobic count in H-3 if needed. Save all dilutions for Enrichment step (see below).

Spread the inoculum using a sterile spreader; use a new spreader for each dilution. Let the MLA medium absorb the inoculum before inverting and incubating the plates for 48 h at 30 ± 2°C. (Tip: To facilitate quick absorption of the inoculum, dry the MLA plates 48 h at 30°C before use.)

Count the colonies from each aerobic plate containing 0.1 ml of the 10^{-1} to 10^{-3} dilutions and record the numbers. For each set of plates with a total of 1 ml of 10-1 dilution, add the number of colonies and record it and do the same for the duplicate set of plates. Calculate and report aerobic plate counts by following the instructions in BAM Chapter 3. *Aerobic Plate Count*, Sections C and D of the Conventional Plate Count Method. Report results as APC/g (ml). If no colonies are obtained on MLA, report the APC as <10 CFU/g (mL).

Enrichment step: Incubate the remaining 10^{-1}, 10^{-2}, and 10^{-3} dilutions in MLB at 30 ± 2°C and examine the MLB enrichments daily for growth. Incubate the enrichments 7 days or shorter if suspected growth is observed. Subculture all enrichments onto both MLA and MacConkey agar plates and incubate the plates 48 h at 30 ± 2°C.

2. Fungi, yeast, and mold plate count (YMPC)

Follow instructions in H-1 for APC to determine the number of fungi by plating on either malt extract agar (MEA) or potato dextrose agar (PDA), each containing 40 ppm chlortetracycline.

After the inoculum is absorbed by the medium, incubate the plates at 30 ± 2°C (Do not invert and do not stack more than 3 plates high).

(Note: the following plate count instructions are based on BAM Chapter 18, *Enumeration of Yeasts and Molds in Food-Dilution Plating Technique*.) Count colonies after 5 days of incubation. If there is no growth after 5 days, re-incubate for another 48 h. Do not remove plates to count colonies before the end of the incubation period because handling of the plates could result in secondary growth from dislodged spores, making final counts invalid. Count colonies on the plates and calculate their numbers as instructed in section H-1, except that the suggested count range is 10-150 colonies. Report as count/g (ml) for yeast and for mold, respectively. If plates from all dilutions have no colonies, report YMPC as <10 CFU/g (mL).

OPTIONAL: For fungal enrichments, dilute the prepared sample decimally in Sabouraud's dextrose broth and incubate as described above for MLB dilutions. If growth occurs, streak on Sabouraud's dextrose agar, MEA, or PDA. The MEA and PDA agars should both contain 40 ppm chlortetracycline.

3. Anaerobic plate count

Perform as described above for APC, using MLA agar, pre- reduced anaerobe agar, and 5% defibrinated sheep blood agar for plating. Incubate blood agar plates in 5-10% carbon dioxide atmosphere (candle jar or CO_2 incubator), and anaerobe agar plates in anaerobic jars. Incubate both for 48 h before counting. Reincubate for 2 more days if no colonies appear at 48 h. Pre-reduce anaerobic agar plates before inoculation by placing them in an anaerobic atmosphere overnight (12-16 h). Incubate anaerobe agar plates in anaerobic atmosphere (anaerobic jar, incubator, or glove box) for 2 days at 35 ± 2°C; incubate MLA plates aerobically for 2 days at 35 ± 2°C as aerobic control. Strict anaerobes will grow only in the anaerobic jars. It is recommended that a small amount (0.1 ml) of inoculum be used to minimize spreading of growth caused by wetness, and that inoculated plates be placed in an anaerobic atmosphere within minutes after inoculation to minimize exposure to oxygen.

Suspected anaerobic organisms must be subcultured aerobically (under CO_2) and anaerobically to establish their true oxygen relationship. Check for terminally located spores in cooked meat broth incubated at 35°C for 2 days. Use of a differential spore stain to detect spores is mandatory. Other methods may detect nonspore artifacts, which could lead to wasted identification efforts. If an obligate anaerobic sporeformer is isolated, consult Dr. Shashi Sharma.

I. Identification of Microbes

Molds and yeasts should be purified and yeasts identified as far as possible using kits, e.g., Vitek yeast card and API yeast assimilation strip. For bacteria, examine all plates and streak morphologically dissimilar colony types onto MacConkey and MLA media. Prepare Gram stain of all morphologically dissimilar colony types obtained in pure culture. With methods given here, isolates may be identified to genus level in general; tests for speciation are listed when necessary. Test results should be evaluated using *Bergey's Manual* (12) or Madden's methods (14). All isolates should be identified to the species level when possible. Commercial identification kits, e.g., API, Roche, Vitek, Hewlett-Packard, are recommended to speciate microbes recovered from enumeration plates and enrichment broth.

1. Gram-positive rods

To enhance sporulation, inoculate starch agar plate with isolate and incubate 48 h at room temperature. Prepare either Gram stain or endospore stain from isolated colony and note position of endospore within vegetative cell (central, terminal, or subterminal), shape of endospore (round or ellipsoidal), and morphology of sporulating cell's sporangium (swollen or not swollen). Test all aerobic sporeforming rods for motility by either of two methods:

Consult refs. 7 and 12 when needed.

a) **Cultivation method**. Stab-inoculate tube of motility test or motility-indole-ornithine medium. Incubate aerobically 18-24 h at room temperature. Growth from line of stab (indicated by turbidity of medium around stab) constitutes a positive test.

b) **Microscopic examination**. Inoculate isolated colony into suitable broth. Incubate aerobically 18-24 h at room temperature. Place one drop of broth culture on clean microscope slide and cover with coverslip. Motility is indicated by individual bacterial cells moving in random directions. Observe at either 400× or under oil immersion.

Further characterization of Gram-positive rods can be done by performing catalase test then following the testing below:

1. If catalase is produced and large bacilli (with spores) are observed, use VITEK BCL card, API 50 CHB or equivalent commercial kit to identify the isolate to the species level, possibly *Bacillus*. When *Bacillus cereus* group is identified as a group, consult BAM Chapter 14, *Bacillus cereus* to further distinguish the species in the group. However, only need to identify Bacillus-like rods when the isolates are recovered from aerobic plates, but not from enrichment.
2. If no catalase is produced, use VITEC CBC card, API 50 CHL or equivalent commercial kit to identify the isolate to the species level, possibly *Lactobacillus.*
3. (Rarely) If catalase is produced and short cocco-bacilli are observed, perform motility test. If an isolate is motile at 20-25°C, non-motile at 37 °C, it is possibly Listeria. Use VITEK GP card, API Listeria or equivalent commercial kit to identify the isolate to the species level.

2. Gram-positive cocci

Streak MLA plate from APC media (MLA or BP), incubate 18-24 h at 35 ± 2°C, and test resultant growth for catalase activity and coagulase production (if catalase-positive).

If no catalase is produced, inoculate bile esculin agar slant, a tube of TSB containing 6.5% NaCl, and a 5% sheep blood agar plate. Incubate 18-24 h at 35 ± 2°C. If organism blackens bile esculin medium and will grow in presence of 6.5% NaCl, report it as "Group D enterococcus" (*Enterococcus* spp.). If it blackens bile esculin medium, but will not grow in presence of 6.5% NaCl, report it as "Group D *Streptococcus*, not enterococcus." If it does not blacken bile esculin medium, report it as either alpha, beta, or gamma hemolytic *Streptococcus*. If 5% sheep blood agar is not available, report it as "*Streptococcus*, not Group D." Perform additional speciation of streptococci if required, using procedures outlined in ref. 7 or serological kits commercially available for this purpose, e.g., Phadebact (Pharmacia Diagnostics, Piscataway, NJ).

If catalase is produced, inoculate the following media with freshly isolated colony: mannitol salt agar, duplicate tubes of oxidative-fermentative (OF) medium with dextrose (overlay 1 tube with sterile vaspar or mineral oil; leave 1 tube loosely capped with no overlay), and enriched agar slant for use in coagulase test. Report organism as *S. aureus* if it is coagulase-positive and/or will ferment mannitol; *S. epidermidis* if it is fermentative as well as oxidative on OF dextrose, is coagulase- negative, and will not ferment mannitol; or *Micrococcus* species if it is oxidative only on OF dextrose.

a. **Catalase test**. Add a drop of 3% H_2O_2 either to isolated colony or to clean microscope slide and place platinum loop carrying some isolate into the drop. Reaction is positive if oxygen gas evolves rapidly (bubble formation). (Nichrome wire loops may give false-positive reactions.) When H_2O_2 is placed directly on a colony the bacteria will be killed. Positive control (*Staphylococcus* or an enteric bacterium) and negative control (*Streptococcus*) should be run concurrently to ensure the quality of the H_2O_2 solution.

b. **Coagulase test**. Inoculate small amount of growth from maintenance slant into 13 × 100 mm tube containing 0.2 ml BHI broth. Incubate 18-24 h at 35 ± 2°C; then add 0.5 ml reconstituted lyophilized rabbit coagulase plasma (with EDTA) and mix thoroughly. Incubate at 35 ± 2°C for 6 h and examine for clotting. Weakly coagulase-producing strains may require overnight incubation for clot formation to be evident. Include known coagulase-positive and known coagulase-negative organism with every set of samples. Consider all strains that yield positive coagulase reaction as *S. aureus*.

3. Gram-negative rods

Inoculate TSI agar slant, MacConkey agar plate, cetrimide agar, and MLA plate with all Gram-negative rods. Incubate 18-24 h at 35 ± 2°C. TSI slant/butt reactions of A/A or K/A (A = acidic; K = alkaline) + H_2S indicate an *Enterobacteriaceae* isolate. K/K, K/NC (NC = no change) or NC/NC reactions indicate nonfermentative (NF) Gram-negative bacilli. If TSI reactions are masked by hydrogen sulfide production, inoculate lactose and glucose carbohydrate broths and incubate 18-24 h at 35 ± 2°C. For an *Enterobacteriaceae* isolate, perf the following tests and use refs. 3, 6, 11-13 to interpret results. The API 20E or equivalent commercial kit may be used to identify to species level. Media necessary for tests are listed in C, above.

If an organism grows on cetrimide agar or is identified as an NF Gram-negative bacillus, determine fluorescent and nonfluorescent pigment production, aerobic production of acid from either glucose, sucrose, xylose, or mannitol, and the production of nitrogen gas from an inorganic nitrogen source; carry out other necessary tests using media listed in D, above. Acetamide utilization, growth at 42°C, and gelatin liquefaction are important tests for distinguishing the three *Pseudomonas* species, *P. aeruginosa*, *P. fluorescens*, and *P. putida*. To interpret results of these tests, use refs. 4, 11, 12, 19 or the API nonfermentative kit and data base. Confirm putative *P. aeruginosa* isolates by the method outlined below.

4. Method for identification of *Pseudomonas aeruginosa*

Identification of *P. aeruginosa* is of particular concern because this organism survives in eye-area cosmetics (21) and has been implicated in eye infections (20). It is opportunistically pathogenic to humans (10) and highly resistant to antibacterial agents such as quaternary ammonium compounds, penicillin, and many broad-spectrum antibiotics.

a) Presumptive identification

TSI agar slants. Transfer well-isolated typical colonies from cetrimide agar plates to TSI agar slants. Streak surface and stab butt. Incubate at 35°C for 24 ± 2 h. All slants having growth and an alkaline (red) slant and alkaline (red) butt should be considered as presumptive positive for *Pseudomonas* spp. and tested for oxidase and other biochemical reactions. Some pseudomonads may produce slight hydrogen sulfide in TSI, but this can be confused with soluble pigments produced by some species.

Oxidase Test

Oxidase test strips (for *Pseudomonas* spp.)	
Tetramethyl-**p**-phenylenediamine-dihydrochloride	1.0 g
Ascorbic acid	0.1 g
Distilled water	100

Cut filter paper (Whatman No. 40) into small strips of about 10 × 40 mm. Shade in reagent. Drain. Spread strips on paper towels on a tray. Shade with paper towels, because light degrades the reagent; dry in 35°C incubator. (Reagent also degrades at higher temperature.) When dry, store in brown bottle at room temperature. Strips must be protected from light and moisture; they should be white. The strips are stable indefinitely.

Use platinum loop to smear mass of cells on portion of strip. (Nichrome wire gives false-positive reactions.) Read at 10 s, **no longer**. Positive is indicated by a deep purple color; negative is indicated by the absence of color or when a purple color appears after 10 s. *Pseudomonas* spp. are oxidase-positive.

b) **Biochemical tests**

Find biochemical tests and test results in a table in the archive.

J. Microbiological Findings

Current, widely accepted standards for microbiological limits in cosmetics specify that the total number of microorganisms per gram or milliliter generally should not exceed:

- $1x10^2$ colony forming units (CFU)/g or mL for cosmetics intended topically (i.e., applied to the surface of the body) for the eye area, mucous membranes, and children <3 years old; and
- $1x10^3$ CFU/g or mL for all other topical cosmetics.

The presence of pathogens would also be important in evaluating the microbial contents of a cosmetic. Pathogens or opportunistic pathogens whose incidence would be of particular concern include *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, and *Candida albicans*. Additionally, microbes normally regarded as nonpathogenic when introduced in certain ways (e.g., topically) may become opportunistic pathogenic and virulent when introduced in other ways (e.g., in wounds, or via cosmetics introduced into or through the skin).

K. Cosmetic preservative efficacy

The above guidelines for interpretation of results apply to cosmetic products before the time of use. Cosmetics contain antimicrobial preservatives and thus are expected to withstand a certain amount of abuse by users. Formerly, there were no validated tests for cosmetic preservative efficacy (9), although the test for pharmaceutical preservative efficacy in the U.S. Pharmacopeia (2) or the cosmetic test in the technical guidelines of the Cosmetic, Toiletry, and Fragrance Association (CTFA) (1) were used. Recently, the CTFA test has been AOAC validated (2b) for use with liquid cosmetics. A test for solid cosmetic preservative efficacy has been proposed (18). Cosmetics in reusable test kits, such as those in retail stores, can be microbiologically evaluated semiquantitatively by a sterile swab test (17).

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