Designation: E 640 – 78 (Reapproved 1998)

Standard Test Method for Preservatives in Water-Containing Cosmetics[1]

This standard is issued under the fixed designation E 640; 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 method covers the determination of the suitability of preservatives for use in cosmetic formulations. It sets minimal requirements for preservative performance in model formulations.

1.2 *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. Summary of Method

2.1 This method involves a microbiological challenge test of preservatives incorporated into formulations at recommended efficacy levels. Routine microbiological procedures are used to determine the antimicrobial activity of preservatives in formulations. This method requires the knowledge of standard microbiological techniques.

3. Significance

3.1 This method should be used to determine if a preservative has application as a cosmetic preservative.

4. Materials

4.1 *Test Formulations*—Formulations that the submitter feels are appropriate for demonstration of preservative activity shall be included in the test. Nonpreserved (control) samples of these formulas shall also be included. Incompatibility of the presevative with any of the formulations or formulation components shall be noted.

4.2 *Test Microorganisms (Minimal Panel)*:

4.2.1 Other test microorganisms shall be included where appropriate and if standardized cultures from cosmetic isolates become available. The primary function of these cultures is to provide a common basis for comparison of different preservatives.

4.2.1.1 *Pseudomonas aeruginosa* ATCC 9027.

4.2.1.2 *Staphylococcus aureus* ATCC 6538.

4.2.1.3 *Candida albicans* ATCC 10231.

4.2.1.4 *Enterobacter aerogenes* ATCC 13048.

4.2.1.5 *Aspergillus niger* ATCC 16404.

4.2.1.6 *Penicillium levitum* ATCC 10464.

4.2.2 Spoilage microorganisms obtained from cosmetics shall be used in addition to the microorganisms described in 4.2.1, if available.

4.3 *Culture Preparation*—The microorganisms listed in 4.2.1 shall be maintained as follows:

4.3.1 *Bacteria*—Nutrient agar (BBL, Difco or equivalent).

4.3.2 *Yeasts and Fungi*—Mycophil agar, pH 4.7 (BBL or equivalent).

4.3.3 Transfer monthly. Incubate bacteria at 32°C and fungi and yeast at 25°C.

4.4 *Media*:

4.4.1 *Nutrient Agar* (BBL or equivalent).

4.4.2 *Letheen Agar* (Difco or equivalent).

4.4.3 *Mycophil Agar*, pH 4.7 (BBL or equivalent).

4.4.4 *Letheen Broth* (Difco or equivalent).

5. Procedures

5.1 *Preparation of Challenge Inocula*—Grow bacterial cultures at 37°C for 18 to 24 h on slants of the appropriate solid media. Grow yeast cultures at 25°C for 48 h. Grow fungal cultures on the appropriate media at 25°C for 7 to 14 days or until full sporulation is achieved.

5.1.1 *Harvesting Bacterial Cultures*—Using a sterile inoculating loop, transfer the growth from each culture into tubes of sterile distilled water. Adjust to an optical density of 0.45 in. ½-in. (12.7-mm) diameter optical quality test tubes using a B&L Spectronic 20 Spectrophotometer (or equivalent) set at 425 nm. This should give approximately 1.0×10^8 bacteria/mL.

5.1.2 *Harvesting Yeast Cultures*—Harvest and adjust yeast cultures as described in 5.1.1. This should give approximately 1.0×10^7 yeasts/mL.

5.1.3 *Harvesting Fungal Cultures and Dislodging Spores*—Harvest fungal cultures and dislodge spores by rubbing the growth gently with a sterile inoculating loop or removing it with a sterile glass impinger. Filter through sterile nonabsorbent cotton. Harvest and adjust the spore level to 1.0×10^7/mL using a hemocytometer.

5.1.4 *Preparation of Inocula*:

[1] This method is under the jurisdiction of ASTM Committee E–35 on Pesticides and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antibacterial and Antiviral Agents.

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