



Designation: E 640 – 78 (Reapproved 1998)

Standard Test Method for Preservatives in Water-Containing Cosmetics¹

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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 preservative 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 *Lethen Agar* (Difco or equivalent).

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

4.4.4 *Lethen 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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5.1.4.1 Mixed Culture Method:

(1) *Gram-Positive Bacteria and Yeasts*—Mix equal portions of gram-positive bacteria and yeast thoroughly and label “Gram-Positive Bacteria and Yeast #1.”

(2) *Gram-Negative Bacteria*—Mix equal portions of gram-negative bacteria and label “Gram-Negative Bacteria #2.”

(3) *Fungi*—Mix equal parts of fungi suspensions thoroughly and label “Fungi #3.”

5.1.4.2 *Pure Culture Method*—Optionally, challenges may also be performed using single (pure) cultures. If this method is chosen, prepare the cultures used for challenges as described in 5.1 and use directly as described in 5.3. Label sample jars appropriately according to the test microorganisms used to challenge that sample.

5.1.5 *Determination of Challenge Inocula Levels*—Prepare serial dilutions of the challenge inocula (5.1.4.1). Plate out in duplicate using Lethen agar. Incubate bacteria and yeast at 32°C for 24 h and fungi at 25°C for 72 h. Determine the number of colony-forming units (cfu) in each inoculum.

5.2 *Sample Preparation*—Weigh out three samples of each formula in 100-g amounts in suitable glass containers (as described herein), and label appropriately.

5.2.1 *Bottle, glass, 4.66 oz.*²

5.2.2 *Closure.*^{2,3}

5.2.3 *Lid, screw-type.*

5.3 *Challenge of Test Formulation*—Challenge each formulation by adding 1 mL of the appropriate inoculum and mixing thoroughly with a sterile nonabsorbent mixing rod (preferably glass), spatula, or suitable mechanical mixer. Store at ambient temperature (20 to 25°C).

5.4 Microbiological Testing:

5.4.1 *Testing for Microbial Level*—Thoroughly mix the test formulation. Prepare a 1+9 (1 part test product plus 9 parts Lethen broth) dilution w/v and mix thoroughly. Prepare additional appropriate 10-fold dilutions in Lethen broth and plate in duplicate using Lethen agar. Incubate bacteria and yeast at 32°C and fungi at 25°C for at least 72 h.

5.4.2 *Test Time Intervals*—Test at 0, 7, 14, 21, and 28 days (additional testings may be performed if appropriate).

5.4.3 Read plates at 72 h and calculate the number of surviving microorganisms per gram of test lotion. Verify the identity of the microorganisms by gram staining where appropriate.

5.5 *Testing for Neutralization of Preservatives*—The presence of an active preservative carried over from the test formulas into the media may inhibit viable microorganisms and result in false negative readings. Test for preservative carryover in the following manner:

5.5.1 If no growth is observed on the dilution plates, streak the 1+9 and 1+99 plates with a mixed culture of gram-negative bacteria (mix equal portions of 1+999 dilutions of 18 to 24-h nutrient broth cultures). A single streak with an inoculating loop across the plate surface is adequate. Perform the same operation with the gram-positive bacterium and yeast, and with a mixed culture of fungi prepared as described in 5.1.4.1. All inocula may be streaked on one or two plates if single streaks are used. Incubate the plates at 32°C and 25°C for 72 h. If growth does *not* occur with any one of the streaks, the preservative was not neutralized and an appropriate neutralizer must be found.

5.6 *Rechallenge*—If the preservative is intended for cosmetics that are subject to repeated insult by the consumer, employ a rechallenge procedure. Rechallenge the lotions at 28 days and continue the test another 28 days, thereby repeating all procedures described for the initial challenge.

6. Interpretation of Data

6.1 The preservative shall show the following activity against test microorganisms that survive in unpreserved test formulations. Appropriate criteria of preservation are:

6.1.1 Gram-positive and gram-negative bacteria and yeasts should show at least a 99.9 % decrease within 7 days following each challenge and no increase thereafter for the remainder of the test within normal variation of the data.

6.1.2 Fungi should decrease by at least 90 % within 28 days and show no increase during the test period within normal variation of the data.

6.2 All nonpreserved controls should fail this test.

NOTE 1—Organisms indigenous to the work place may react differently than the test organisms presented herein.

7. Precision and Bias

7.1 The precision and bias of this method have not been determined. Replicate samples are recommended.

8. Keywords

8.1 bacterial; cosmetics; microbial challenge; preservative performance criteria; preservatives

² The source of supply of the apparatus known to the committee at this time is Owens-Illinois Glass Container Div., Toledo, Ohio. Order No. AW-105 97, 58-405. If you are aware of alternative suppliers, please provide this information to ASTM Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend.

³ The source of supply of the apparatus known to the committee at this time is Owens-Illinois Glass Container Div., Toledo, Ohio. Order No. 43601, MM No. 14261, 58-400.