Journal of Medical Microbiology and Infectious Diseases

JoMMID
ISSN: 2345-5349
eISSN: 2345-5330

Microbiological Assessment of Commercial Perfumes from Different Supply Chains in Iran Using the Direct Inoculation Method of Pharmacopeial Sterility Tests

Sayyed Behnam Farmanara[1], Mostafa Pournamdari[2], Shahram Shoeibi[3], Mohammad Hassan Moshafi[4, 5], Maryam Kazemipour[6], Ali Asadipour[7], Mehdi Ansari Dogaheh[4, 5]*

[1]Student Research Committee, Kerman University of Medical Sciences, Kerman, Iran; [2]Herbal and Traditional Medicines Research Center, Institute of Pharmaceutical Sciences, Kerman University of Medical Sciences, Kerman, Iran; [3]Food and Drug Laboratory Research Center, Food and Drug Administration, Iranian Ministry of Health and Medical Education, Tehran, Iran; [4]Pharmaceutics Research Center, Institute of Pharmaceutical Sciences, Kerman University of Medical Sciences, Kerman, Iran; [5]Department of Drug and Food Control, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran; [6]Department of Chemistry, Faculty of Sciences, Islamic Azad University, Kerman Branch, Kerman, Iran; [7]Pharmaceutical Sciences and Cosmetic Products Research Center, Institute of Pharmaceutical Sciences, Kerman University of Medical Sciences, Kerman, Iran

ARTICLE INFO

Original Article

Keywords: Ethanol self-preservation, Polysorbate 80 neutralization, USP <71>, Direct inoculation method, Cosmetic microbiology

Received: 23 May. 2026
Received in revised form: 29 Jun. 2026
Accepted: 07 Oct. 2026
DOI: [To be assigned]

***Correspondence**
Email: mansari@kmu.ac.ir, mansari1345@yahoo.com

© The Author(s)

ABSTRACT

Introduction: The microbiological integrity of perfumes is essential for safety, stability, and consumer trust. Although the high alcohol content is assumed to be self-preserving or antimicrobial against vegetative cells, comparative empirical data across different supply chains remain limited. This study aimed to assess the absence of detectable viable growth in perfumes from diverse market sources using pharmacopeial sterility test conditions. **Methods:** A total of 97 finished perfume samples were categorized as counterfeit/unauthorized (n=25), officially imported (n=15), and domestically manufactured (n=57). Sterility testing was performed by direct inoculation into Fluid Thioglycollate Medium (FTM) and Tryptic Soy Broth (TSB) according to USP <71>. The media were supplemented with 0.5% *v/v* polysorbate 80 to neutralize the antimicrobial activity of the alcohol. FTM vials were incubated at 25 ± 1°C and TSB vials at 35 ± 1°C for 14 days. Turbid vials underwent subculturing on Tryptic Soy Agar (TSA) and Potato Dextrose Agar (PDA). **Results:** No detectable viable microbial growth was observed under the test conditions. Eight samples (8.2%) developed slight, non-progressive turbidity, but confirmatory subculturing on TSA and PDA yielded no colonies after 72 hours, suggesting that the turbidity resulted from physicochemical interactions rather than viable microbial contamination. **Conclusion:** This analysis of 97 samples suggests that finished perfumes from the evaluated supply chains in Iran were free of detectable viable growth under the test conditions; USP <71> was applied as an investigative tool rather than as a regulatory sterility requirement for cosmetic products. The results support the inherent antimicrobial properties of the high-alcohol matrix and, where applicable, the effectiveness of existing manufacturing controls. They also underscore the value of routine confirmatory subculture protocols during microbiological investigations of high-alcohol cosmetic products to avoid false-positive batch failures.

INTRODUCTION

The global fragrance industry represents a multibillion-dollar market in which product integrity, consumer safety, and brand reputation are inextricably linked [1-3]. Perfumes are complex mixtures of aromatic compounds, solvents (primarily ethanol), and fixatives, and represent cosmetic products intended for direct and prolonged contact with human skin [4-6]. Consequently, their microbiological quality is a critical parameter influencing not only product stability and shelf life but also, more importantly, consumer health and safety [6-8].

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

Microbial contamination in cosmetics can originate from raw materials, water, manufacturing equipment, the production environment, or packaging [9, 10]. For perfumes, the high alcohol content (typically 70–95% ethanol) is widely presumed to confer intrinsic self-preservation, creating a hostile environment for most vegetative bacteria, yeasts, and molds [8, 11, 12]. Previous studies have consistently demonstrated that ethanol concentrations above approximately 70% exert broad-spectrum antimicrobial activity against vegetative microorganisms [11, 12]. Modern self-preserving cosmetic systems often combine multifunctional ingredients such as phenethyl alcohol and caprylyl glycol, which exhibit broad-spectrum antimicrobial activity without being classified as traditional preservatives [13]. This presumption often leads to a perceived lower risk profile compared with water-based cosmetics; however, it does not eliminate the risk entirely, because contamination with alcohol-tolerant microorganisms, bacterial or fungal spores, or process deviations during manufacturing and filling can occur [14, 15].

Counterfeit or unauthorized cosmetic products may be manufactured outside regulated Good Manufacturing Practice (GMP) environments and may involve uncontrolled raw materials, non-validated water systems, and inadequate hygienic controls, potentially increasing microbiological risk [16]. The consequences of microbial contamination are multifaceted. From a product integrity standpoint, microbial metabolism can degrade delicate fragrance compounds, alter pH, cause turbidity or sediment formation, and produce off-odors, ultimately rendering the product unacceptable [17-19]. From a public health perspective, contaminated products applied to skin, particularly compromised or sensitive skin, can cause irritations, allergic reactions, or infections [20, 21]. A comprehensive review by Lundov *et al.* highlights the delicate balance between product preservation and the risk of preservative-induced contact allergy [21].

To mitigate these risks, regulatory frameworks worldwide mandate stringent quality control measures. Compendial and regulatory standards, such as those outlined in the United States Pharmacopeia (USP), European Union cosmetic legislation, and International Organization for Standardization (ISO) guidelines, provide benchmarks for microbial-limit testing, preservative-effectiveness testing, and GMP compliance [22-26]. While full sterility is not a regulatory requirement for non-sterile cosmetics, including perfumes, demonstrating the absence of specified pathogens and acceptable levels of non-pathogenic microorganisms is essential. Sterility testing according to USP <71> remains the reference method for assessing the absence of viable microorganisms in sterile products and can serve as an investigative tool for high-risk batches or specific consumer complaints [26-28]. Although perfumes are not required to be sterile, evaluating the absence of detectable viable microorganisms using the USP <71> direct inoculation procedure may provide useful insight into their microbiological status and the applicability of this compendial method to the perfume matrix [26].

Despite the economic and safety significance of perfumes, there is a notable paucity of large-scale, systematic studies in the scientific literature that empirically evaluate their microbial quality across diverse market segments [19, 29, 30]. Most existing research focuses on water-based cosmetics (*e.g.*, lotions and creams) [9, 15, 29, 30]. Data comparing the microbiological status of products from different supply chains—counterfeit/unauthorized sources, officially licensed imports, and domestically manufactured items—are particularly scarce. Such comparative analysis is crucial because supply-chain integrity is a key determinant of adherence to GMP [8, 9, 31].

This study was designed to address this knowledge gap. The primary objective was to conduct a comprehensive, compendial-based assessment of a large cohort of perfume samples (n=97) systematically collected from three distinct sources: counterfeit/unauthorized markets, official import channels, and domestic production lines. By applying the rigorous direct inoculation method per USP <71> with appropriate antimicrobial neutralization, this investigation aimed to: 1) provide empirical data on the microbiological status of finished perfume products; 2) compare microbial quality indicators across different supply chains; and 3) verify the suitability of standard sterility-testing methodologies for this specific, high-alcohol matrix [32-34]. The findings contribute valuable evidence to inform quality control practices, regulatory oversight, and risk assessment in the fragrance industry.

MATERIAL AND METHODS

Study design and sample collection. A total of 97 finished perfume samples were collected for this analytical study. Samples were purposively selected to represent three predefined market supply-chain categories: Category 1, counterfeit/unauthorized (n=25; sample IDs 101–125), comprising products lacking standard manufacturer identification that were purchased from unofficial vendors and transit markets; Category 2, officially imported (n=15; sample IDs 1–15), comprising products sourced from official distributors with verifiable international brand origins; and Category 3, domestically manufactured (n=57; sample IDs 201–257), comprising products obtained directly from licensed national manufacturers. Within the imported and domestically manufactured categories, individual products were randomly procured from licensed pharmacies and authorized distributors, whereas counterfeit/unauthorized samples, identified by the absence of standard manufacturer labeling, batch codes, and holographic seals, were purchased from

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

unauthorized street vendors in Tehran and Kerman. The original perfume bottles ranged in volume from 30 mL to 100 mL. All samples were stored at controlled room temperature in their original packaging until analysis, which was initiated within 48 hours of receipt.

Sterility testing: culture media preparation and quality control. Sterility testing was performed based on the direct inoculation procedure described in the current USP General Chapter <71> "Sterility Tests" [26]. Two primary liquid culture media were employed to support the growth of a wide spectrum of microorganisms:

a) Fluid Thioglycollate Medium (FTM; Condalab, Spain): Prepared at a concentration of 29.8 g/L in double-distilled water. This medium is designed for the cultivation of aerobic and anaerobic bacteria, including fastidious anaerobes.

b) Tryptic Soy Broth (TSB; QueLAB, Canada): Prepared at a concentration of 30 g/L in double-distilled water. This medium supports the growth of non-fastidious bacteria, yeasts, and molds.

To neutralize the inherent antimicrobial activity of the high alcohol content in perfume samples, 0.5% *v/v* polysorbate 80 (Tween® 80, Merck, Germany) was added to the medium before dispensing and autoclaving [32-34]. Polysorbate 80 acts as a surfactant to emulsify hydrophobic perfume components, while dilution of a 1.0 mL inoculum in 20 mL of medium reduces the ethanol concentration below inhibitory levels (typically < 5% *v/v* in the final broth matrix) [11, 12, 32, 34].

The media were gently heated in a boiling water bath to ensure complete dissolution. Subsequently, 20 mL aliquots were dispensed into sterile 50 mL borosilicate glass vials sealed with aluminum crimp caps. Sterilization was achieved by autoclaving at 121°C (15 psi) for 20 minutes. All media batches underwent rigorous quality control: they were incubated at 35–37°C for 72 hours prior to use to confirm the absence of intrinsic microbial contamination. Growth-promotion capability was evaluated separately during method suitability testing [35].

Sample inoculation and incubation. The direct inoculation method was employed for sterility testing. Under a Class II biosafety cabinet with strict aseptic technique, 1.0 mL of each undiluted perfume sample was aseptically transferred into a vial of FTM and, separately, 1.0 mL into a vial of TSB using a sterile barrier-tip micropipette. The vials were gently agitated to mix and then incubated under two temperature regimes to maximize recovery: FTM vials were incubated at 25 ± 1°C and TSB vials at 35 ± 1°C for 14 days. Note that, in contrast to the compendial specifications of USP <71> (FTM, 30–35°C; TSB, 20–25°C), the incubation temperatures assigned to the two media were deliberately exchanged so that each medium was evaluated under a different thermal regime and the overall detection spectrum was broadened; this deviation from the compendial incubation conditions is acknowledged as a limitation of the study [26]. This dual-temperature approach was intended to facilitate the recovery of both mesophilic (35°C) and potential environmental or slow-growing (25°C) microorganisms.

Controls. To validate the entire testing system, the following controls were included:

a) Negative media control: Uninoculated vials of FTM and TSB from each batch were incubated alongside test samples to confirm medium sterility.

b) Positive growth control: To verify the ability of the culture media to support microbial growth, positive growth controls were established using *Escherichia coli* (ATCC 8739) and *Bacillus subtilis* (ATCC 6633). Inocula of no more than 100 CFU of each microorganism were introduced separately into sterile TSB and FTM media containing 0.5% polysorbate 80. The inoculated media were incubated under the same conditions applied to the test samples and examined daily for evidence of microbial growth.

Method suitability. Method suitability was evaluated to determine whether residual antimicrobial activity of the perfume matrix could inhibit microbial recovery. Representative perfume samples were inoculated into TSB and FTM media containing 0.5% polysorbate 80 and subsequently challenged with ≤100 CFU of *E. coli* (ATCC 8739) and *B. subtilis* (ATCC 6633). Recovery and visible growth of the challenge microorganisms following incubation demonstrated that the neutralization procedure was sufficient to prevent inhibition of microbial growth under the conditions of this study [26, 32-34].

Environmental monitoring. All procedures were conducted in a controlled environment. Work surfaces and vial exteriors were disinfected with 70% ethanol and 10% povidone-iodine (Betadine®) before transfer into the cabinet to reduce cross-contamination.

Examination and interpretation of results. All inoculated vials were examined daily for visual signs of microbial growth, including turbidity, pellet formation, surface pellicle, or gas production. Samples showing any visual turbidity or ambiguity at any point during the 14-day incubation were subjected to confirmatory testing.

Confirmatory subculturing for turbid samples. For any vial exhibiting turbidity, a subculture was performed immediately. Using a sterile loop, a sample from the turbid broth was streaked onto two solid media:

a) Tryptic Soy Agar (TSA): For the isolation of bacteria and yeasts.

b) Potato Dextrose Agar (PDA): For the enhanced isolation of molds and yeasts.

TSA plates were incubated at 30-35°C, whereas PDA plates were incubated at 20-25°C for a minimum of 72 hours, and the plates were examined daily for colony formation. The absence of growth on solid media

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

indicated that the observed turbidity in the liquid medium was not of microbial origin.

Data analysis. A sample was classified as "no confirmed growth" if neither the FTM nor the TSB vial showed any evidence of growth throughout the 14-day incubation period. If turbidity occurred but subculturing on TSA and PDA yielded no microbial growth after 72 hours, the result was also recorded as "no confirmed growth". Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Fisher's exact test was used to compare turbidity frequencies among supply-chain categories. Statistical significance was defined as $P < 0.05$.

RESULTS

Sterility testing outcomes. Throughout the 14-day incubation period, all uninoculated negative control media remained clear, confirming the absence of intrinsic contamination in the culture media (Figure 1). In contrast, positive growth controls demonstrated the expected microbial proliferation. *E. coli* in TSB showed visible turbidity at 24 ± 2 hours, and *B. subtilis* in TSB showed visible turbidity at 36 ± 4 hours (Figure 2). In FTM, *E. coli* showed turbidity at 36 ± 6 hours, and *B. subtilis* showed turbidity at 48 ± 2 hours (Figure 3). All perfume samples remained free of detectable microbial growth under the study conditions. A representative sample showing no growth (Sample 64) is indicated in Figure 3D.



Fig. 1. Representative negative controls during the 14-day incubation period. Uninoculated FTM and TSB vials after completion of incubation.



Fig. 2. Representative positive controls during the 14-day incubation period, showing turbidity: *E. coli* in TSB at 24 ± 2 hours; *B. subtilis* in TSB at 36 ± 4 hours.

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

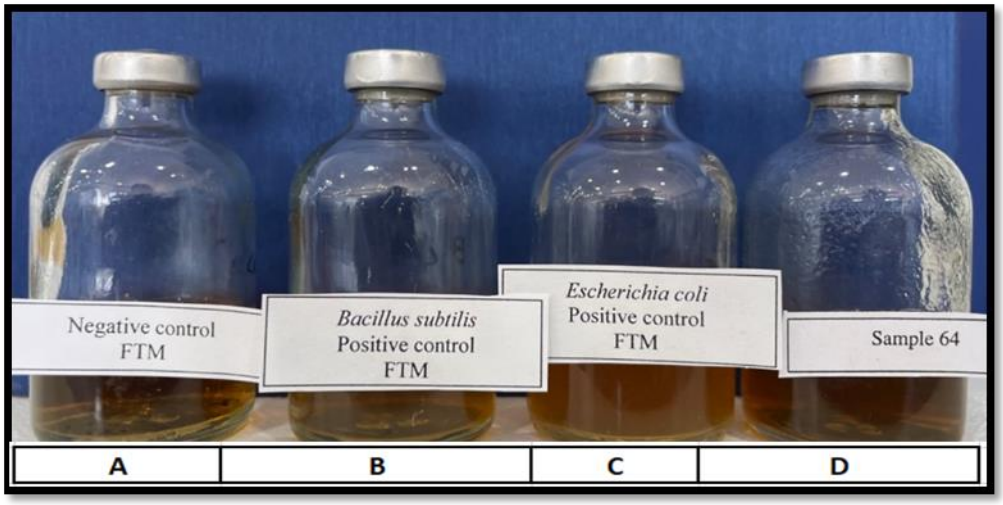


Fig. 3. Representative control and test cultures used during the 14-day incubation period: A. Uninoculated Fluid Thioglycollate Medium (negative control) after completion of incubation; B. Positive growth control inoculated with *Bacillus subtilis* ATCC 6633; C. Positive growth control inoculated with *Escherichia coli* ATCC 8739; D. Representative perfume sample (Sample 64) following incubation under the study conditions.

Analysis of turbidity events and confirmatory subculturing. During the daily examination, a subset of samples (8 out of 97, representing 8.2% of the total) presented with slight, non-progressive turbidity in either TSB, FTM, or both media. The turbidity appeared as a uniform haziness without flocculent precipitation, pellicle formation, or bottom sediment, and did not increase in intensity over time. These samples were identified and immediately subjected to the confirmatory subculturing protocol as stipulated in USP <71>. The details of these samples are presented in Table 1.

Table 1. Characteristics and confirmatory testing results of perfume samples exhibiting turbidity in sterility test media

Sample ID	Product category	Turbidity observed in media*	Day of first observation	Subculture media used **	Subculture incubation (72 h)	Final interpretation
101	Counterfeit/unauthorized	TSB & FTM	Day 4	TSA, PDA	No growth	No confirmed growth
105	Counterfeit/unauthorized	TSB	Day 7	TSA, PDA	No growth	No confirmed growth
5	Imported	FTM	Day 5	TSA, PDA	No growth	No confirmed growth
204	Domestic	TSB	Day 3	TSA, PDA	No growth	No confirmed growth
222	Domestic	TSB & FTM	Day 6	TSA, PDA	No growth	No confirmed growth
227	Domestic	TSB	Day 5	TSA, PDA	No growth	No confirmed growth
236	Domestic	TSB & FTM	Day 4	TSA, PDA	No growth	No confirmed growth
249	Domestic	TSB	Day 2	TSA, PDA	No growth	No confirmed growth

Abbreviations: *FTM, Fluid Thioglycollate Medium; TSB, Tryptic Soy Broth. **TSA, Tryptic Soy Agar; PDA, Potato Dextrose Agar.

As detailed in Table 1, subculturing from the turbid broth onto solid culture media (TSA and PDA) and subsequent incubation for 72 hours yielded no microbial colony formation on any plate. This critical step confirmed that the observed turbidity was not of microbiological origin. The absence of growth on both general-purpose (TSA) and fungus-supporting (PDA) media does not prove absolute sterility, but it argues against the presence of viable bacteria, yeasts, and molds recoverable under these conditions. The turbidity was therefore attributed to physicochemical interactions between specific components of the perfume formulations and the constituents of the liquid culture media, potentially exacerbated by the presence of the neutralizing agent polysorbate 80 [37-43].

Categorical summary of results. The overall sterility test results, stratified by the sample source category, are summarized as follows:

Counterfeit/unauthorized samples (n=25): All 25 samples showed no confirmed microbial growth. Two

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

samples (8.0% of this category) showed non-microbial turbidity but were confirmed free of recoverable growth via subculture.

Imported samples (n=15): All 15 samples showed no confirmed microbial growth. One sample (6.7% of this category) showed non-microbial turbidity but was confirmed free of recoverable growth via subculture.

Domestically manufactured samples (n=57): All 57 samples showed no confirmed microbial growth. Five samples (8.8% of this category) showed non-microbial turbidity but were confirmed free of recoverable growth via subculture.

Across all 97 samples, the rate of confirmed absence of recoverable growth was 100% under the test conditions, and the incidence of non-microbial turbidity was 8.2%. Comparison across product categories was performed using Fisher's exact test due to small expected frequencies in certain subgroups. No statistically significant association was observed between supply-chain category and turbidity occurrence (two-sided Fisher's exact test, $P = 0.933$; Table 2), indicating that turbidity occurrence was not concentrated in any category and was more consistent with formulation-dependent physicochemical interference than with category-related manufacturing quality.

Table 2. Distribution of turbidity events by supply chain category

Category	Total Samples (n)	Turbidity Observed (n)	Percentage (%)	Confirmed Microbial Growth	Final Status
Counterfeit/unauthorized	25	2	8.0	0	No confirmed growth
Imported	15	1	6.7	0	No confirmed growth
Domestic	57	5	8.8	0	No confirmed growth
Total	97	8	8.2	0	No confirmed growth

DISCUSSION

The results of this extensive sterility testing study demonstrate a consistent outcome: all 97 perfume samples, spanning three distinct market categories (counterfeit, imported, and domestic), were free of detectable viable growth under the USP <71> direct-inoculation conditions applied here. This absence of growth, together with the caveat that strict anaerobes, some fungi, and injured or slow-growing organisms may require specialized culture conditions not fully replicated in this protocol, provides empirical support for the intrinsic antimicrobial efficacy of the standard perfume formulation matrix, which is predominantly based on high concentrations of ethanol or other alcohols [8, 11, 12]. The uniform outcome across categories is a particularly notable finding. It suggests that even products from unofficial or "counterfeit" supply chains, which might be expected to bypass formal quality controls, did not show microbial contamination in this assay. This may be attributable to the intrinsic preservative property of the high-alcohol content itself, which acts as a formidable barrier to microbial survival and proliferation during formulation and filling, although it does not exclude contamination by alcohol-tolerant organisms or spores under other conditions [14-16, 31]. For imported and domestically manufactured perfumes, the results are consistent with the effectiveness of established GMP in the fragrance industry [25, 31]. These findings align with the theoretical expectation of microbial stability in such products but provide the empirical laboratory evidence that has been lacking in the scientific literature [19, 29, 30].

A critical methodological insight from this work pertains to the interpretation of turbidity in sterility test media. In a limited number of samples (Table 1), slight turbidity developed in one or both media. However, confirmatory subculturing onto TSA and PDA yielded no microbial growth, arguing against contamination. The absence of a statistically significant association between supply-chain category and turbidity occurrence (Fisher's exact test, $P = 0.933$) indicates that turbidity was not clustered across counterfeit, imported, and domestic samples. This finding suggests that turbidity was not related to manufacturing quality or GMP compliance, because products from unauthorized sources would be expected to show higher rates of true contamination if GMP deviations were the primary cause. Instead, consistent with challenges noted in growth-based sterility testing evaluations [36], the distribution across all three categories points to a formulation-dependent phenomenon. Certain hydrophobic perfume ingredients (*e.g.*, aromatic oils, resins, or fixatives) may form fine micelles or precipitates when mixed with aqueous culture media, thereby producing turbidity even in the absence of viable organisms [36-43]. This interpretation is further supported by the observation that turbidity appeared non-progressively on different days (ranging from day 2 to day 7) and did not increase in intensity over time, which is inconsistent with microbial growth (typically visible within 24–48 hours and progressive) [26, 27]. This observation carries significant practical importance for quality control microbiology. It underscores the paramount necessity of adhering to pharmacopeial protocols that mandate subculture from turbid sterility test media. Relying solely on visual

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

inspection of broth tubes could lead to false-positive sterility test failures, resulting in unnecessary product rejection, costly investigations, and production delays [26-28, 35]. Our study provides a clear case example of this non-microbial turbidity in cosmetic product testing. The successful neutralization of alcohol by the addition of 0.5% polysorbate 80 to the culture media was a crucial factor in ensuring the test's validity. Without this step, the residual bacteriostatic effect of alcohol transferred with the inoculum could have suppressed the growth of low-level contaminants, leading to false-negative results [32-34, 44, 45]. The effectiveness of the neutralization strategy was confirmed qualitatively in two steps. First, both *E. coli* and *B. subtilis* showed robust growth in FTM and TSB containing 0.5% polysorbate 80 without perfume samples, confirming that the neutralizing agent alone did not inhibit microbial recovery (growth-promotion control). Second, both reference strains also grew robustly in media containing 0.5% polysorbate 80 and representative perfume samples, confirming method suitability, *i.e.*, that residual components of the perfume matrix did not suppress microbial recovery after neutralization. However, because method suitability was verified with only two bacterial strains, recovery of strict anaerobes, yeasts, and molds was not fully confirmed under the conditions of this study [26]. The dual incubation temperatures (25°C and 35°C) further strengthened the test by creating conditions suitable for mesophilic and potential environmental contaminants, ensuring a broad spectrum of detection, but did not replace a full compendial validation panel [26, 35].

From a regulatory and safety perspective, these findings are reassuring for finished high-alcohol perfumes applied to intact skin. The primary microbial quality concerns for such products therefore shift toward the following: 1) the quality of raw materials (especially water and non-alcoholic extracts) before alcohol addition, 2) the hygiene of manufacturing processes to prevent gross contamination, and 3) the integrity of packaging to maintain microbiological control during shelf life and after consumer opening [14, 15, 20, 25]. Our study focused on the finished-product absence-of-growth endpoint, which appears to be well controlled. Accordingly, the results should not be interpreted as advocating routine sterility testing for perfumes. International cosmetic quality assurance generally emphasizes ISO 22716 GMP, microbial limits according to ISO 17516 or USP <61>/<62>, and preservative-effectiveness testing according to ISO 11930 or USP <51> where applicable [22-25, 46-48]. USP <71> was used here as a sensitive investigative tool, not as a regulatory sterility requirement for cosmetic products.

Limitations of this study should be acknowledged. First, the study design was a "snapshot" in time and did not evaluate microbial stability over the entire shelf life of the products or after repeated consumer use, which introduces water and skin microbiota [20, 21]. Second, despite the large sample size, the geographical sourcing might not be fully representative of the global market. Third, method suitability verification was conducted using only two bacterial strains (*E. coli* and *B. subtilis*). In contrast, full validation according to USP <71> requires a panel of at least six index microorganisms, including anaerobes (*Clostridium sporogenes*), yeasts (*Candida albicans*), and molds (*Aspergillus brasiliensis*) [26]. Consequently, the ability of the test system to support the recovery of strict anaerobes and fungi was not confirmed under the conditions of this study, and the incubation temperatures used here deviated from the compendial specifications (FTM at 25°C instead of 30–35°C; TSB at 35°C instead of 20–25°C). Fourth, method suitability assessment was based on qualitative growth recovery rather than quantitative recovery ratios and therefore represents a partial verification of neutralization effectiveness. Fifth, alcohol concentration, pH, and specific formulation components were not measured, limiting mechanistic attribution of the turbidity. Sixth, the 1.0 mL inoculum per medium represents only a small fraction of the container volume; consequently, very low-level contamination present in the bulk product could escape detection. Finally, the absence of growth under these conditions does not prove absolute sterility or the absence of non-viable microbial residues.

Future research directions emerging from this work include: 1) investigating the specific chemical interactions between perfume ingredients and culture media to better predict or prevent non-microbial turbidity, 2) conducting challenge tests to determine the efficacy of perfume formulations against specific resilient spores, 3) exploring rapid microbiological methods as potential alternatives for high-throughput screening [27, 36], and 4) performing longitudinal studies on microbial contamination dynamics in perfumes after opening and consumer use [20, 21]. Such studies would complement the present finished-product assessment and strengthen risk-based cosmetic microbiological control.

In conclusion, no detectable viable microorganisms were recovered from the 97 commercially available perfume samples tested under the conditions of the USP <71> direct inoculation procedure. These findings support the well-recognized microbiological stability of high-alcohol perfume formulations while emphasizing that USP <71> was used in this study as an investigative microbiological tool rather than as a regulatory sterility requirement for cosmetic products. From a quality assurance and regulatory perspective, microbiological control of perfumes should continue to focus primarily on GMP, microbiological quality of raw materials before alcohol incorporation, microbial limits testing (USP <61>/<62> or ISO 17516), and preservative efficacy testing where appropriate (USP <51> or ISO 11930),

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

rather than routine sterility testing of finished products [22-25, 46-48]. The principal practical contribution of this study is the demonstration that slight, non-progressive turbidity observed during pharmacopeial sterility testing of perfumes should always be evaluated by confirmatory subculturing in order to distinguish physicochemical interference from true microbial contamination and thereby avoid false-positive interpretations.

ACKNOWLEDGMENT

The authors thank Kerman University of Medical Sciences for providing laboratory facilities and technical support for this study.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript**.**

FUNDING

This study received no financial support.

DATA AVAILABILITY

The data presented in this study are available on reasonable request from the corresponding author.

AI DISCLOSURE

During the preparation of this work, Grammarly and QuillBot were used to improve language and readability. The authors reviewed and edited all suggestions and took full responsibility for the final content.

AUTHORS' CONTRIBUTIONS

SBF: Methodology, Formal Analysis, Writing - Original Draft. MP: Methodology, Writing - Review & Editing, Supervision, Conceptualization, Investigation. SS: Formal Analysis, Writing-Review & Editing. MHM: Methodology, Writing-Review & Editing, Supervision. MK: Writing-Review & Editing, Formal Analysis, Methodology. AA: Conceptualization. MAD: Conceptualization, Methodology, Investigation, Writing-Review & Editing, Supervision.

ETHICS STATEMENT

The study protocol was registered and approved by the Ethics Committee of Kerman University of Medical Sciences (IR.KMU.REC.1404.101).

REFERENCES

1. Rocha F, Ratola N, Homem V. Fragrances in the environment: properties, applications, and emissions. In: Ratola N, Homem V, editors. Fragrances in the environment. Cham: Springer; 2024. p. 1–36.

2. Liaqat I, Jurrat H, Ali NM, Aftab MN, Ali S, Naseem S, et al. Cosmeceutical legislation: global and Pakistan view. In: Javed S, Abrar S, Arshad M, editors. Sustainable cosmeceuticals: integrating natural and biotechnological innovations. Cham: Springer; 2025. p. 479–506.

3. Gebashe FC, Naidoo D, Amoo SO, Masondo NA. Cosmeceuticals: a newly expanding industry in South Africa. Cosmetics. 2022;9(4):77.

4. Nakano AK. Understanding fragrance: from chemistry to emotion. In: Benson HAE, Roberts MS, Leite-Silva VR, Walters KA, editors. Cosmetic formulation: principles and practice. Boca Raton: CRC Press; 2019. p. 263–277.

5. Sharmeen JB, Mahomoodally FM, Zengin G, Maggi F. Essential oils as natural sources of fragrance compounds for cosmetics and cosmeceuticals. Molecules. 2021;26(3):666.

6. Chisvert A, López-Nogueroles M, Miralles P, Salvador A. Perfumes in cosmetics. In: Salvador A, Chisvert A, editors. Analysis of cosmetic products. 2nd ed. Amsterdam: Elsevier; 2018. p. 225–248.

7. Alblooshi S. The impact of perfumes and cosmetic products on human health: a narrative review. Front Toxicol. 2025;7:1646075.

8. Halla N, Fernandes IP, Heleno SA, Costa P, Boucherit-Otmani Z, Boucherit K, et al. Cosmetics preservation: a review on present strategies. Molecules. 2018;23(7):1571.

9. da Silva JD, Silva FAM, Rodrigues CF. Microbial contamination in cosmetic products. Cosmetics. 2025;12(5):198.

10. Alshehrei FM. Microbiological quality assessment of skin and body care cosmetics by using challenge test. Saudi J Biol Sci. 2024;31(4):103965.

11. Oh E, Choi SJ, Han S, Lee KH, Choi H-J. Highly effective salt-activated alcohol-based disinfectants with enhanced antimicrobial activity. ACS Nano. 2023;17(18):17811–25.

12. Maillard J-Y, Pascoe M. Disinfectants and antiseptics: mechanisms of action and resistance. Nat Rev Microbiol. 2024;22(1):4–17.

13. Herman A. Antimicrobial ingredients as preservative booster and components of self-preserving cosmetic products. Curr Microbiol. 2019;76(6):744–54.

14. Clegg A, Perry BF. Control of microbial contamination during manufacture. In: Baird RM, Bloomfield SF, editors. Microbial quality assurance in cosmetics, toiletries and non-sterile pharmaceuticals. 2nd ed. Basingstoke: Taylor & Francis; 1996. p. 49–66.

15. Michalek IM, John SM, Caetano dos Santos FL. Microbiological contamination of cosmetic products—observations from Europe, 2005–2018. J Eur Acad Dermatol Venereol. 2019;33(11):2151–7.

16. Kim HW, Seok YS, Cho TJ, Rhee MS. Risk factors influencing contamination of customized cosmetics made on-the-spot: evidence from the national pilot project for public health. Sci Rep. 2020;10(1):1561.

17. Choi E, Maeng SJ, Yun S, Yu H, Shin J-S, Yun J-Y. The degeneration of skin cosmetics and the structural changes of the chemical components as an indicator of product shelf life. J Ind Eng Chem. 2021;100:317–23.

18. Eleni P, Kalouta K, Panagiotou N, Vassilatou K, Krokida M. Preparation, quality characterization, and shelf-life

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]

evaluation of facial cosmetic cream enriched with encapsulated tea tree oil. J Cosmet Sci. 2022;73(2):70–83.

19. Feng Y, Zhang T, Yang J, Liu W, Yang Y, Huang J, et al. Characterization of microbial communities in flavors and fragrances during storage. Front Microbiol. 2025;16:1516594.

20. Campana R, Scesa C, Patrone V, Vittoria E, Baffone W. Microbiological study of cosmetic products during their use by consumers: health risk and efficacy of preservative systems. Lett Appl Microbiol. 2006;43(3):301–6.

21. Lundov MD, Moesby L, Zachariae C, Johansen JD. Contamination versus preservation of cosmetics: a review on legislation, usage, infections, and contact allergy. Contact Dermatitis. 2009;60(2):70–8.

22. European Parliament, Council of the European Union. Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products. Official Journal of the European Union. 2009;L 342:59–209.

23. International Organization for Standardization. ISO 17516:2014 Cosmetics — Microbiology — Microbiological limits. Geneva: ISO; 2014.

24. International Organization for Standardization. ISO 11930:2019 Cosmetics — Microbiology — Evaluation of the antimicrobial protection of a cosmetic product. Geneva: ISO; 2019.

25. International Organization for Standardization. ISO 22716:2007 Cosmetics — Good Manufacturing Practices (GMP) — Guidelines on good manufacturing practices. Geneva: ISO; 2007.

26. United States Pharmacopeial Convention. General chapter <71>: Sterility Tests. In: United States Pharmacopeia–National Formulary (USP 48–NF 43). Rockville, MD: USPC; 2025.

27. Marsit NM, Saadawi S, Alennabi K. Challenges of growth-based microbiological methods in sterility assurance of pharmaceutical product manufacturing. Discov Pharm Sci. 2025;1(1):13.

28. England MR, Stock F, Gebo JET, Frank KM, Lau AF. Comprehensive evaluation of compendial USP <71>, BacT/ALERT Dual-T, and BACTEC FX for detection of product sterility testing contaminants. J Clin Microbiol. 2019;57(2):e01548-18.

29. Almukainzi M, Alotaibi L, Abdulwahab A, Albukhary N, El Mahdy AM. Quality and safety investigation of commonly used topical cosmetic preparations. Sci Rep. 2022;12(1):18299.

30. Stewart SE, Parker MD, Amézquita A, Pitt TL. Microbiological risk assessment for personal care products. Int J Cosmet Sci. 2016;38(6):634–45.

31. Chejor P, Dorji T, Dema N, Stafford A. Good manufacturing practice in low- and middle-income countries: challenges and solutions for compliance. Public Health Chall. 2024;3(1):e158.

32. Eldemerdash A, Ewaisha R, Alseqely M, Shehat MG. Optimizing neutralization strategies for microbial testing of non-sterile pharmaceutical finished products with challenging method suitability. BMC Microbiol. 2025;25(1):748.

33. Gorsuch JP, Jones Z. Customized antimicrobial efficacy tests offer superior evaluation of growth inhibitor efficacy for liquid microbial products. Heliyon. 2020;6(2):e03419.

34. Katerji A, Trefi S, Bitar Y, Ibrahim A. Evaluation of new formulations for neutralizing antimicrobial preservatives in pharmaceutical preparations. Heliyon. 2023;9(3):e14555.

35. Weenk GH, van den Brink J, Meeuwissen J, van Oudenallen A, van Schie M, van Rijn R. A standard protocol for the quality control of microbiological media. Int J Food Microbiol. 1992;17(2):183–98.

36. Parveen S, Kaur S, David SA, Kenney JL, McCormick WM, Gupta RK. Evaluation of growth based rapid microbiological methods for sterility testing of vaccines and other biological products. Vaccine. 2011;29(45):8012–23.

37. Kanei N, Tamura Y, Kunieda H. Effect of types of perfume compounds on the hydrophile-lipophile balance temperature. J Colloid Interface Sci. 1999;218(1):13–22.

38. Tchakalova V. Preferential solubilization of fragrances in micelles with different geometric shapes. In: Mejuto JC, Poša M, editors. Design and applications of self-assembly aggregates—from micelles to nanoemulsions. London: IntechOpen; 2024. p. 63–84.

39. Sikora E, Miastkowska M, Wolinska-Kennard K, Lason E. Nanoemulsions as a form of perfumery products. Cosmetics. 2018;5(4):63.

40. Vitale SA, Katz JL. Liquid droplet dispersions formed by homogeneous liquid–liquid nucleation: the ouzo effect. Langmuir. 2003;19(10):4105–10.

41. Dallay C, Malhiac C, Picard C, Savary G. Fragrance in dermocosmetic emulsions: from microstructure to skin application. Int J Cosmet Sci. 2024;46(1):1–23.

42. Linke C, Drusch S. Turbidity in oil-in-water emulsions—key factors and visual perception. Food Res Int. 2016;89(Pt 1):202–10.

43. Venkataramani D, Tsulaia A, Amin S. Fundamentals and applications of particle stabilized emulsions in cosmetic formulations. Adv Colloid Interface Sci. 2020;283:102234.

44. Brauge T, Chalivat E, Leleu G, Colas A, Midelet G. Impact of disinfectant-neutralizing buffers used for sampling methods on the viability of adherent *Listeria monocytogenes* cells on surfaces. FEMS Microbiol Lett. 2025;372:fnaf048.

45. United States Pharmacopeial Convention. General chapter <1227>: Validation of microbial recovery from pharmacopeial articles. In: United States Pharmacopeia–National Formulary (USP 48–NF 43). Rockville, MD: USPC; 2025.

46. United States Pharmacopeial Convention. General chapter <51>: Antimicrobial Effectiveness Testing. In: United States Pharmacopeia–National Formulary (USP 48–NF 43). Rockville, MD: USPC; 2025.

47. United States Pharmacopeial Convention. General chapter <61>: Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests. In: United States Pharmacopeia–National Formulary (USP 48–NF 43). Rockville, MD: USPC; 2025.

48. United States Pharmacopeial Convention. General chapter <62>: Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms. In: United States Pharmacopeia–National Formulary (USP 48–NF 43). Rockville, MD: USPC; 2025.

Cite this article:
Farmanara SB, Pournamdari M, Shoeibi S, Moshafi MH, Kazemipour M, Asadipour A, Ansari Dogaheh M. Microbiological Assessment of Commercial Perfumes from Different Supply Chains in Iran Using the Direct Inoculation Method of Pharmacopeial Sterility Tests. J Med Microbiol Infect Dis, 2026; 14 (3): 221-229.

[Downloaded from jommid.pasteur.ac.ir on 2026-11-16]