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Assessment of Airborne Microbial Contamination in Cosmetics Manufacturing Facilities: Skincare Cream Production in Thailand

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Abstract

Skincare is a variety of practices to maintain skin integrity, enhance appearance, and alleviating skin conditions. Thailand's skincare market has seen substantial growth, becoming the dominant sub-sector in cosmetics. Microbial contamination in the skincare can occur when the manufacturing process are not well controlled. The objective of this research is to establish criteria for managing appropriate levels of microbial quantities in the air of Good Manufacturing Practice (GMP) cosmetics production facilities. Airborne microbial samples are collected using both active air sampler and settle plate techniques at varying time intervals, followed by a comparative analysis. The skincare creams as representatives showed that the total airborne microbial counts using the air sampling method, ranged from 85 to 252 cfu/m^3. For the settle plate method for 4 and 1 hours ranged from 8 to 90 cfu/4h and 1 to 59 cfu/h. The action limits from control charts at 341 cfu/m^3, 107 cfu/4h, and 59 cfu/h for the respective methods. Based on the results of this research, it can be concluded that the monitoring criteria for cosmetics manufacturing facilities, with a specified limit for airborne microbial counts not exceeding 100 cfu/4h (sterile medicinal products at Grade D) or 50 cfu/h (moderate IMA level).

Keywords : airborne microbial contamination; cosmetics; good manufacturing practice (GMP); indoor air quality; microbial environmental monitoring; monitoring criteria

Introduction

Cosmetic products, as non-sterile health products, require production in clean environments to prevent contamination risks that can compromise product quality, consumer safety, and industry reputation. Although not subjected to the same aseptic standards as vaccine production, high-risk cosmetic products are susceptible to contamination, which can have repercussions on both health and product quality.

Skincare encompasses a range of practices designed to preserve skin integrity, enhance its appearance, and address various skin conditions such as lotions, facial creams, eye creams, sunscreens, skin serums, hair and scalp treatments, and non-colored lip balms. The skincare market in Thailand has witnessed substantial growth in recent years, establishing itself as the predominant sub-sector within the cosmetic industry.

To prevent microbial cross contamination during production, it is imperative to maintain the cleanliness of five key sources: water, raw materials, equipment, personnel, and the environment [1]. FDA data from 2004-2011 reveal that 31% of cosmetic product recalls were attributed to microbial contamination, predominantly the Pseudomonads group, with *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) accounting for 34% of cases. This marked an increase from the 22% prevalence observed during 1998-2006 [2]. Furthermore, between 2002 and 2016, there were 313 cosmetic recalls, with the majority linked to bacterial contamination. These recalls included 14 level 1 recalls (indicating severe health risks), 266 level 2 recalls (associated with temporary health effects), and 33 level 3 recalls (with no significant health concerns) [3].

Due to these global concerns, regulations such as Good Manufacturing Practice (GMP) have gained significance in ensuring the safety and quality of health products. These guidelines focus on controlling personnel hygiene, production facilities, equipment, environmental conditions, manufacturing processes, quality control, and storage.

Microbiological standards for cosmetics establish limits, with a maximum total microbial count (including yeast and mold) of 1000 cfu/g or ml. Prohibited microorganisms in 1 g or 1 ml include *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus*, and *Candida albicans* [4]. Cosmetics are categorized under various classifications, such as skincare, foundation, powder, hair color, and fragrance products [5]. The risk associated with these products depends on factors such as pH, alcohol content, hydrogen peroxide, filling temperature, and water activity, as detailed in ISO 29621 [6] guidelines for low-risk products. High-risk products, such as skincare creams, can remain susceptible to contamination during production, even within controlled environments, highlighting the necessity for microbiological environmental monitoring.

Criteria of cleanroom for pharmaceutical production

Microbiological Environmental Monitoring (EM) is a means of demonstrating acceptable microbiological quality in a controlled environment and detecting changes in time. It involves collecting data on microbial counts recovered from air, surfaces, and people in pharmaceutical production area. EM describes the microbiological testing for evaluating the cleanliness of manufacturing environments of both sterile and nonsterile products [7]. In the context of sterile pharmaceutical control, EM encompasses four tests: active air samples or volumetric sampling, settle plates, contact plates, and fingerprint sampling during cleanroom monitoring. The limits for microbial EM listed in Table 1 apply exclusively to sterile pharmaceuticals, such as vaccines. These standards serve the purpose of prequalification, distinguishing grades A, B, C, and D, where "A" denotes operations in at-risk areas, "B" for sterile areas, "C" for control areas, and "D" for support areas [8-9].

Table 1 Microbiological cleanliness levels in operation

Grade	Air Sample (cfu/m^3)	Dia. 90 mm. Settle Plate (cfu/4h)	Dia. 55 mm. Contact Plate (cfu/plate)	Glove print (cfu/glove)
A	< 1	< 1	< 1	< 1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

Cleanroom environments are established based on ISO 14644-1 and ISO 14698-1 standards to ensure cleanliness and control [10], with the Pharmaceutical and Healthcare Sciences Society [11] consolidating several cleanroom standards. The microbiological limits in cleanrooms are typically classed as follows: Class 100000, comparable to ISO 8 and EU Grade D, with values of 200 CFU/m^3 or settle plates (90 mm) at 100 CFU/4 hours; Class 10000, similar to ISO 7 and EU Grade C, with values of 100 CFU/m^3 or settle plates (90 mm) at 50 CFU/4 hours; and Class 100, which aligns with ISO 6,5 and EU Grade A and B.

Another criterion, as proposed by Pasquarella et al. [12], is the index of microbial air contamination (IMA), which is based on microbial fallout counts on Petri dishes exposed to the air according to the 1/1/1 scheme (for 1 hour, 1 meter from the floor, at least 1 meter away from walls or any obstacles). IMA is classified into five categories: 1) very good: 0-5; 2) good: 6–25; 3) fair: 26–50; 4) poor: 51–75; and 5) very poor: > 76.

However, there is currently a lack of specific microbial contamination control criteria for cosmetics or non-sterile health product manufacturing facilities. This absence of standardized control criteria has left manufacturers without reliable benchmarks. Utilizing criteria from pharmaceuticals may not be suitable for cosmetics due to variations in product characteristics. Implementing stringent control measures such as fumigation to eliminate contaminants within production areas can be costly and disruptive. In contrast, the food industry provides guidelines for microbiological laboratory working areas, setting a limit of no more than 15 cfu/15 minutes (FDA–BAM, 2001).

Salaman [13] advises manufacturers to create their own suitable control criteria by assessing the risks associated with microbial cross-contamination in the environment. Subsequently, control criteria for microbes can be developed through the generation of statistical control charts. Furthermore, Pitzurra et al. [14] recommend generating scientific data to select dependable and appropriate methods tailored to the facility's needs. Validation and verification of these methods should precede implementation. Additionally, it is advisable to establish Standard Operating Procedures (SOPs) concerning environmental microbial sampling, data interpretation, and ensuring precise and consistent understanding among personnel.

This research-intensive approach can pose challenges for microbiologists in industrial settings, as it entails routine work and significant responsibilities. These challenges can be quite demanding for microbiologists in the industry, typically employed in laboratories as quality control or quality assurance officers. Their workload is generally characterized by routine tasks and more.

The objective of this study is to establish criteria for managing appropriate levels of airborne microbial contamination in Good Manufacturing Practice (GMP) cosmetics production facilities to ensure product quality and safety, with a focus on skincare cream production.

Materials and Methods

Selection of Sampling Sites

To comprehensively analyze microbial contamination within the cosmetic cream manufacturing sector, five manufacturing facilities in Thailand were chosen. During the selection process, careful attention was given to identifying high-risk zones characterized by direct interaction between the air and products or raw materials. These zones included areas such as weighing rooms, mixing rooms, semi-product storage rooms, filling rooms, and packaging rooms.

We selected five skincare cream manufacturing facilities certified under the ASEAN Cosmetic GMP guidelines [15], representing the cosmetics industry. The choice to emphasize skincare cream manufacturing is rooted in the significant expansion of the skincare market in Thailand, where skincare has emerged as the dominant sub-sector among all cosmetic categories. The susceptibility of skincare products to microbial contamination arises from insufficient control measures during both the manufacturing process and storage.

Sampling Techniques and Equipment

A multifaceted approach to airborne microbial sampling was employed, utilizing various techniques and equipment: Volumetric Air Sampling - One cubic meter (1 m^3) of air within the manufacturing chambers was

collected using the Sampl'Air Lite device by BIOMERIEUX [8, 16]. Settle Plate Method (4-Hour Exposure) - Standard Petri dishes with a 90 mm diameter were used [8]. Settle Plate Method (1-Hour Exposure, per the Index of Microbial Air Contamination, IMA) - following the 1/1/1 scheme, involving placement 1 meter from the floor and at least 1 meter away from walls or any relevant physical obstacles. Petri dishes with a 90 mm diameter were employed [12].

Environmental parameters that could influence the study outcomes, including personnel count, temperature & humidity [thermo-hygro-meter], and wind speed [anemometer], were measured and recorded.

Culture and Analysis

TSA (Tryptic Soy Agar, [HIMEDIA]) and DG-18 ([HIMEDIA]) were used for bacterial and fungal analysis, respectively [17]. MacConkey Agar ([HIMEDIA]) was employed for the cultivation of Gram-negative bacteria [18]. Bacterial sample agar plates underwent incubation at 35°C for 48 hours, while fungal culture plates were incubated at 25°C for 5-7 days.

Microbial Identification

Following incubation, each sample underwent meticulous examination to determine the presence or absence of colony forming units (CFU). Bacterial identification involved Gram's staining and morphological characterization, while fungal species were identified based on colony and hyphal morphology, with the assistance of staining using lactophenol blue.

The 16S rRNA gene analysis was used for identifying bacterial isolates. The amplification and sequencing of the 16S rRNA gene were performed by Macrogen (Seoul, South Korea) using universal primers [19]. BLAST was performed using the EzBiocloud 16S database [20].

Risk assessment

To conduct a risk assessment for microbial contamination, we will assess the "likelihood" and "impact" of contamination. Likelihood is associated with the contamination rate from the airborne environment, expressed as a percentage of the contamination rate (% CR). Meanwhile, the impact pertains to the results of total microbial and Gram-negative bacterial counts that the cosmetic product is from each production room, as presented in Table 2

Table 2 The five levels of impact from the the microbial count and types

Score Level	Microbial count and types (CFU)	Impact Level
1	Total count <10 & No Gram -	Negligible
2	Total count 10-100 & No Gram -	Minor
3	Total count 100-500 & No Gram -	Moderate
4	Total count 500-1000 and/or found Gram -	Major
5	Total count >1000 and/or found Gram -	Critical

To evaluate this, we will create a risk matrix based on the likelihood-impact relationship, using the % CR values to generate likelihood tables for contamination at five levels, as presented in Table 3. The likelihood levels are determined by referencing the IMA table, and the maximum value in each IMA table range is used to calculate the contamination rate (%CR) according to Sandle's Numerical Approaches to Risk Assessment [7] as follow;

$$\%\,CR = \text{Settle plate count} \times \frac{\text{Area of product}}{\text{Area of Petri-dish}} \times \frac{\text{Time product exposure}}{\text{Time settle plate}} \times 100$$

%CR calculation is based on the surface area of the product, determined by the cross-sectional area of the cream container (4 cm) and the surface area of the agar plate (9 cm). The product's exposure to air lasts 1 minute, while the agar plate is exposed for 1 hour, following the IMA method.

Table 3 The five likelihood levels of microbial contamination

Likelihood Level	IMA value	% Contamination rate (%CR)	Score Level
Very good	0 - 5	< 2 %	1
Good	6 - 25	>2 - 8 %	2
Fair	26 - 50	>8 - 16 %	3
Poor	51 - 75	>16 - 25 %	4
Very poor	> 75	> 25 %	5

We can multiply each likelihood and impact level to establish the risk matrix shown

in Table 4. The microbial contamination risk can be estimated from the risk matrix. This matrix contains five colored boxes. The red boxes are very high-risk, the orange boxes are high-risk, the yellow boxes are moderate-risk, the light blue boxes are low risk, and the green boxes are very low-risk.

Table 4 The risk matrix by multiplying likelihood and impact

Matrix	Impact level					
Likelihood Levels	Risk Rating	Negligible	Minor	Moderate	Major	Critical
	Frequent	5	10	15	20	25
	Probable	4	8	12	16	20
	Occasional	3	6	9	12	15
	Remote	2	4	6	8	10
	Improbable	1	2	3	4	5

The control chart calculation

The Shewhart Control Chart Method [21] involves establishing statistical control criteria by sampling microbial data. Typically, 20-30 consecutive samples are collected to construct a variable control chart. The mean and standard deviation (SD) are calculated to set the warning limit at mean ± 2SD and the action limit at mean ± 3SD, providing a robust framework for monitoring and controlling contamination risks.

Results and Discussion

Airborne Microbial Assessment in Cosmetics Manufacturing Factories

1. Factory 1: skincare creams and powder

Microbial counts in the air at critical points within Factory 1 were sampled in 9 rooms. It was found that the total microbial count in the air, using the air sampling method, ranged from 87 to 252 cfu/m^3 (colony-forming units per cubic meter). Count placed on agar plates for 4 hours ranged from 9 to 49 cfu/4h, and those placed on agar plates for 1 hour ranged from 1 to 18 cfu/h, as shown in Table 5. The fungal count in the air, using the same air sampling method, ranged from 87 to 203 cfu/m^3 and agar plates for 4 hours, ranged from 10 to 45 cfu/4h. The gram-negative bacterial count in the air, using agar plates for 4 hours, ranged from 0 to 5, as shown in Table 7.

It was observed that the total microbial count in the air in all rooms was relatively low when compared to the microbial control standards for cosmetics. However fungal counts obtained from air sampling were also high (87 to 203 cfu/m^3) which is above the ISO 7218 [22] recommendation that suggests using agar plates with a diameter of 90 mm for fungal counts with a range between 10-150 cfu (for bacteria, the range should be between 10-300 cfu). Therefore, in the subsequent research within the factory, the fungal testing was discontinued using the air sampling method.

2. Factory 2: skincare creams

Microbial counts in the air at critical points within Factory 2 were sampled in 5 rooms. It was found that the total microbial count in the air, using the air sampling method, ranged from 105 to 240 cfu/m^3. Count placed on agar plates for 4 hours ranged from 25 to 90 cfu/4h, and those placed on agar plates for 1 hour ranged from 14 to 41 cfu/h, as shown in Table 5. The fungal count in the air on agar plates for 4 hours, ranged from 18 to 74 cfu/4h. The gram-negative bacterial count in the air, using agar plates for 4 hours, ranged from 0 to 1, as shown in Table 7.

3. Factory 3: skincare, mascara, hair dye

Microbial counts in the air at critical points within Factory 3 were sampled in 14 rooms. It was found that the total microbial count in the air from skincare and makeup products, using the air sampling method, ranged from 108 to 270 cfu/m^3. Count placed on agar plates for 4 hours ranged from 26 to 164 cfu/4h, and those placed on agar plates for 1 hour ranged from 4 to 58 cfu/h.

The airborne microbial assessment of hair dye products, conducted using the air sampling method, revealed a range of 262 to 400 cfu/m^3. The counts placed on agar plates for 4 hours varied from 134 to 701 cfu/4h, while those placed on agar plates for 1 hour showed a range of 52 to 100 cfu/h, as detailed in Table 3.

Observations were carried out in the Hair Care Mixing A room, designated for hair dye mixing. The total microbial count in this room reached as high as 701 cfu/4h and 100 cfu/h. This elevation in microbial counts can be attributed to the use of water spray within the room to regulate temperature, resulting in a substantial increase in airborne contamination levels. However, the air

sampling method yielded a lower count of 400 cfu/m^3. This variance may be associated with the air sampling technique, which entails drawing air through approximately 300 holes.

The fungal count in the air on agar plates for 4 hours, ranged from 5 to 110 cfu/4h. The gram-negative bacterial count in the air, using agar plates for 4 hours, ranged from 0 to 91, as shown in Table 7.

4. Factory 4: skincare creams

The results of microbial analysis in the air within Factory 4 which only skincare creams products were examined in 6 rooms. It was found that the total microbial count in the air, using the air sampling method ranged from 85 to 240 cfu/m^3, agar plates for 4 hours and 1 hour ranged from 8 to 34 cfu/4h. and 1 to 26 cfu/h, respectively as shown in Table 5. The fungal count in the air, using agar plates incubated for 4 hours, ranged from 3 to 17 cfu. The gram-negative bacterial count in the air, using agar plates incubated for 4 hours, ranged from 0 to 3.

5. Factory 5: skincare creams and powder

The results of microbial analysis in the air within Factory 5, were examined in 7 rooms. It was found that the total microbial count in the air, using the air sampling method and agar plates incubated for 4 hours and 1 hour, ranged from 124 to 242 cfu/m^3, 37 to 73 cfu/4h, and 16 to 59 cfu/h, respectively. as shown in Table 5. The fungal count in the air, using agar plates incubated for 4 hours, ranged from 28 to 74 cfu, and no gram-negative bacteria were detected in the air.

From all the data, Factory 4 had the lowest microbial count, with production room walls and ceilings made of ISOWALL and PU (Polyurethane) flooring, both of which are easy to clean. The next lowest microbial count was found in Factory 1, where, despite using smooth concrete or partition walls, the facility was well-managed and maintained in terms of cleanliness.

The comparison of results from airborne microbial examinations across 41 rooms in five Cosmetics Manufacturing Factories showed that using the air sampler with the European Union Good Manufacturing Practice (EU GMP) method [8], and a settle plate for 4 hours with the EU GMP method, and a settle plate for 1 hour with the IMA method, yielded mean values of 194 cfu/m^3, 84 cfu/4h, and 28 cfu/h, respectively. The corresponding standard deviations (SD) were 84 cfu/m^3, 116 cfu/4h, and 24 cfu/h. Elevated SD values, surpassing the mean, raise concerns about data reliability. Individual factory analysis revealed that Factory 3 had significantly higher levels of total microbes, gram-negative bacteria, and fungi compared to others. To identify outliers, a Huge Error method was applied, uncovering values exceeding 4 for all hair care production rooms and some skincare production rooms.

Consequently, an additional risk assessment was conducted to validate the reliability of the test results, focusing on product types: skincare, makeup, and hair care products. Each production area for different cosmetic product groups was distinctly separated according to their respective categories. The outcomes of this risk assessment led to the following conclusions:

Risk assessment for cosmetics plant

The results of the risk assessment for the cosmetics manufacturing plant indicate that, during the risk identification survey of the production area in Factory 3, it was found that this factory produces skincare, makeup, and hair care products. Samples of airborne microbes were collected in 14 risk rooms, which are rooms directly in contact with raw materials and products, as illustrated in the factory layout in Figure 1

According to Table 6, the raw material weighing and mixing rooms in Factory 3 pose the highest risk probability, reaching level 5. Similarly, three packing rooms also exhibit the highest risk probability, yet the microbial quantities for each product in these rooms are minimal (less than 10 cfu/g), with no gram-negative bacteria, resulting in negligible impact levels. The risk matrix by multiplying likelihood and impact, places the overall risk levels between 1 and 3, deemed acceptable (see Table 4).

The comprehensive analysis of airborne microbial contamination risk in Factory 3 indicates the production of various cosmetic groups, spanning skincare, makeup, and hair care. Notably, the hair care group has comparatively lower cleanliness standards, resulting in a medium risk level.

Table 5 Comparison method of air sampling, settle plates 4 hours and 1 hour from 5 cosmetics factories

Factory	Room	Air Sampler (cfu/m^3)	90 mm. Settle Plate (cfu/4h)	90 mm. Settle Plate (cfu/h)
1	Raw Material Weighing	222	20	N/D
	Cream Mixing	87	13	2
	Skin Cream Filling	252	49	18
	Dust Powder Mixing	121	9	4
	Dust Powder Filling	115	18	5
	Cake Powder Mixing	116	14	1
	Powder Grinding	124	40	15
	Powder Sieving	105	32	15
	Cake Powder Pressing	91	30	10
2	Raw Material Weighing	162	84	32
	Cream Mixing Room	240	60	41
	Semi-product Storage	105	25	14
	Skin Cream Filling F1	156	54	14
	Skin Cream Filling F2	195	90	36
3	Raw Mat. Weighing YK	135	81	24
	Raw Mat. Weighing NB	197	103	36
	Skin Care Mixing D	270	42	16
	Cream Filling A	250	151	49
	Skin Care Filling K	208	104	37
	Skin Care Filling L	135	164	58
	Make up Filling M	161	38	25
	Make up Filling N	108	26	4
	Raw Mat. Weighing Fl.1	368	229	66
	Hair Care Mixing A	400	701	100
	Hair Care Mixing B	350	205	52
	Hair Care Mixing H	265	134	61
	Hair Care Filling C	262	222	71
	Hair Care Filling D	391	199	69
4	Raw Mat. Weighing 1	240	32	16
	Raw Mat. Weighing 2	219	18	9
	Cream Mixing	85	8	1
	Bulk & Labelling	194	34	2
	Cream Filling	127	9	6
	Assembly	95	34	26
5	Raw Mat. Weighing	235	54	21
	Drying	124	52	24
	Cream Mixing	212	73	59
	Semi-product Storage	241	37	22
	Cream Filling	208	60	28
	Packaging & Labelling	242	56	25
	Storage	137	44	16
	Average / Mean	194	84	28
	Standard Deviation	84	116	24

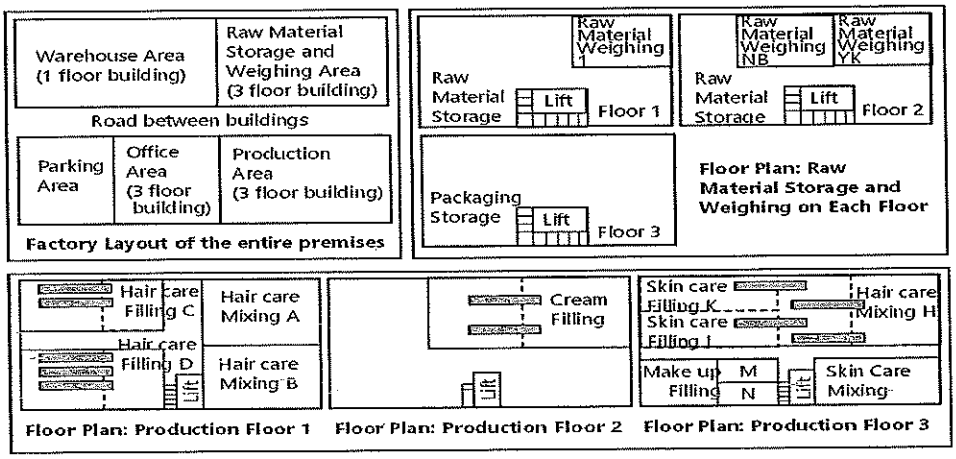

Figure 1 Layout of Factory 3 – Cosmetic Production plant skincare, mascara, hair dye

Table 6 Contamination rate values (% CR) for all 14 risk rooms in Factory 3 to determine the likelihood level

Room in Factory 3	Settle Plate (cfu/h)	Area of product	Time product exposure	% CR	Likelihood level
Raw Mat.Weigh YK	24	79	2	99	5
Raw Mat.Weigh NB	36	79	2	148	5
Skin Care Mixing	16	50	3	63	5
Cream Filling	49	7	1	9	3
Skin Care Filling K	37	0.5	1	19	4
Skin Care Filling L	58	20	1	30	5
Make up Filling M	25	7	1	5	2
Make up Filling N	4	1	1	0.1	1
Raw Mat. Weigh 1	66	79	2	272	5
Hair Care Mixing A	100	7	3	395	5
Hair Care Mixing B	52	50	3	205	5
Hair Care Mixing H	61	39	1	62	5
Hair Care Filling C	71	39	1	72	5
Hair Care Filling D	69	7	1	13	3

Table 7 The environment on microbial quantities in the air

Factory	Temperature (C)	Relative Humidity (%)	Wind speed (km/h)	Total Microbial Count (cfu/4 h)	Fungi (cfu/4 h)	Gram Neg. (cfu/4 h)
1	23.3 – 33.5	36.1 – 63.5	0 – 1.7	9 - 69	10 - 45	0 - 5
2	25.6 – 30.6	59.7 – 71.9	0 – 2.0	25 - 90	18 - 74	0 - 1
3	18.4 – 31.2	46.5 – 66.8	0 – 1.9	8 - 701	5 - 110	0 - 91
4	21.1 – 28.8	45.1 – 56.0	0 – 0.8	8 - 34	3 - 17	0 - 3
5	19.7 – 25.5	40.5 – 58.9	0 – 0.1	37 - 73	28 - 74	0

The influence of the environment on microbial quantities in the air

The results of microbial testing in the air of each room were examined in relation to the environmental conditions to assess the influence of the environment on microbial quantities in the air, as detailed in Table 7. It was found that environmental factors such as temperature, humidity, and wind speed in each room of all five factories did not show a significant correlation with the quantity of microbes in the air. Generally, the temperature in each room, measured in each factory, ranged from 20-33°C, which is a normal temperature range for microbial growth. Meanwhile, the relative humidity in the air generally ranged between 40-60%, with some rooms having humidity exceeding 60%, posing a higher risk of mold formation. Rooms with humidity below 40% were drier, and moisture from agar plates could be released into the air, potentially affecting the microbial assessment results [23].

Regarding wind speed within the rooms, the measured values were generally low. Continuous sampling throughout the year revealed that temperature and relative humidity influenced higher concentrations of airborne fungi during the rainy season, aligning with the findings of studies conducted in chocolate factories [24] and in post-harvest fruit environments [25]. These studies demonstrated that temperature and humidity played a role in increased airborne fungal concentrations during the rainy season.

The classification of microbiology in the air of cosmetic manufacturing

The classification of microbial types in the air of cosmetic manufacturing factories 1 to 5 revealed variations among the factories. The percentages for Gram-positive cocci were 78, 62, 63, 75, and 3%; Gram-positive rods were 7, 16, 16, 15, and 3%; Gram-negative bacteria were 13, 2, 4, 9, and 0%. As for molds, the percentages were 2, 20, 1, 1, and 93%. Additionally, Gram-positive filamentous bacteria were found to be 1%. A detailed comparison of microbial classification for each cosmetic factory in Figure 2.

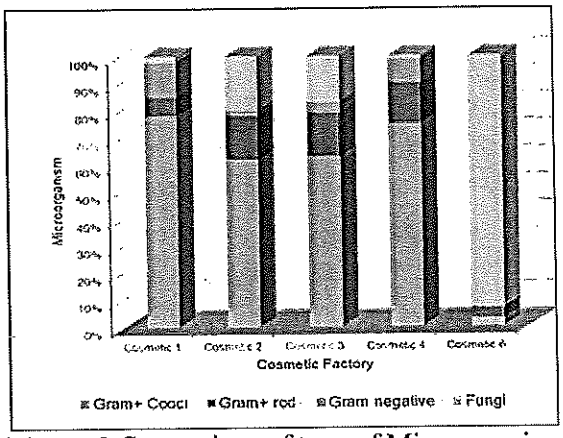

Figure 2 Comparison of type of Microorganism

In general, the predominant bacteria found in most factories were Gram-positive cocci, ranging from 62% to 78%. These bacteria are commonly present on human skin. Factory 5, however, showed a dominance of fungi as the primary microorganisms, reflecting the influence of external air where molds are prevalent (299 cfu/4h or 87%). Despite efforts to control microbial levels, Factory 5 continued to have proportions similar to those found outside.

These research findings align with the study conducted by Sandle [26], which investigated bacteria in cleanrooms with over 9000 samples over 9 years. The study found that Gram-positive cocci, originating from human skin, accounted for 81%, 63%, and 41% in Grade A-B, C, and D rooms, respectively. Gram-positive rods, sourced from soil, followed, and Gram-negative bacteria, derived from water and raw materials, were also identified. Fungi (only molds) constituted 1-8%, with higher prevalence in humid conditions [27].

The identification of bacteria and fungi in the air of cosmetic manufacturing

The results of sequencing the 16S rRNA gene show that the bacteria can be classified at the species level, as indicated in Table 8. Additionally, the fungi were classified at the genus level based on morphology of the conidia using a light microscope. All detected bacteria are non-prohibited types in cosmetics.

Table 8 Identification of microbial contaminants in cosmetics manufacturing factories

Microbial types	Genus / Species
Gram-positive cocci	*Brachybacterium muris*, *Kocuria rhizophila*, *Macrococcus brunensis*, *Micrococcus luteus*, *Mammaliicoccus sciuri*, *Staphylococcus argenteus*, *Staphylococcus caprae*, *Staphylococcus haemolyticus*, *Staphylococcus ureilyticus*, *Staphylococcus hominis* subsp. *Hominis*
Gram-positive rod	*Bacillus cereus*, *Bacillus paramycoides*, *Bacillus paranthracis*, *Bacillus siamensis*, *Bacillus pumilus*, *Bacillus siamensis*, *Brevibacterium casei*, *Cytobacillus firmus*, *Exiguobacterium acetylicum*, *Mesobacillus thioparans*, *Priestia aryabhattai*
Gram-positive filamentous bacteria	*Streptomyces parvulus*
Gram-negative bacteria	*Acinetobacter baumannii*, *Pantoea stewartii* subsp. *indologenes*, *Pseudomonas stutzeri*, *Pseudomonas oleovorans* subsp. *oleovorans*, *Stutzerimonas stutzeri*
Fungi	*Penicillium* sp., *Aspergillus* sp.

Guidelines for controlling microbes in the air of cosmetics manufacturing

In situations where there are no explicit regulations or standards defining microbial control parameters for the air within cosmetics manufacturing factories, statistical calculations become necessary. For example, the Shewhart Control Chart Method [21] can be employed.

Based on microbial air assessment data from four skincare cosmetics manufacturing factories (a total of 27 samples), mean values were calculated as 18 cfu/h, 39 cfu/4h, and 165 cfu/m^3, with corresponding standard deviations of 14, 23, and 59, respectively. Utilizing these values, warning limits for each method were determined as 45 cfu/h, 84 cfu/4 h, 282 cfu/m^3. Action limits were set at 59 cfu/h, 107 cfu/4h, 341 cfu/m^3, as illustrated in Figure 3. This approach aligns with Salaman [13] suggesting manufacturers can establish suitable control criteria by evaluating the risk of environmental cross-contamination and comparing continuous microbial assessment results with risk assessment reports. Subsequently, control criteria can be defined through statistical control chart creation, considering warning and action levels for existing bioburden in water for pharmaceutical production [28].

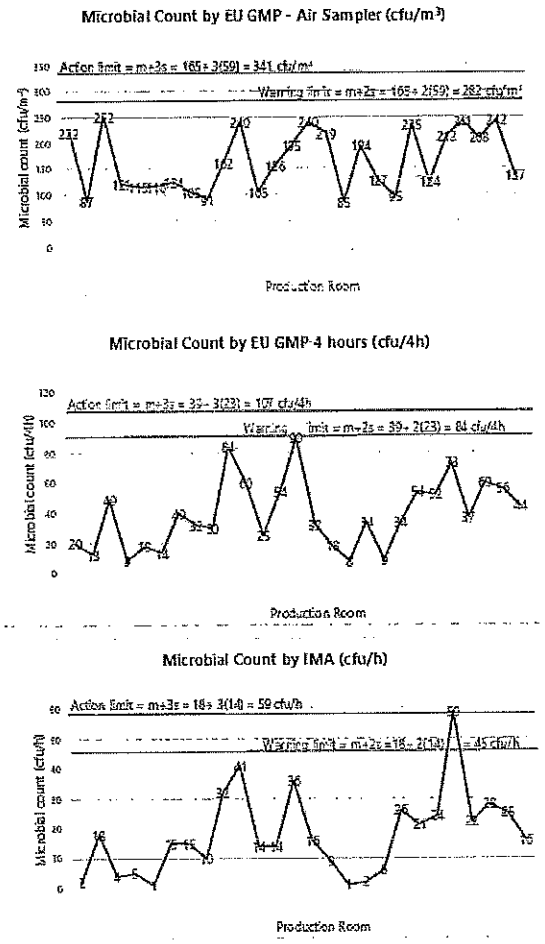

Figure 3 Shewhart Control Chart of air sampling, settle plates 4 hours and 1 hour from skincare cream rooms.

Adopting guidelines from EU GMP [8], the airborne microbial assessments conducted in each skincare factory revealed total microbial counts ranging from 8 to 90 cfu/4h. These values consistently remained below 100 cfu/4h. According to the EU GMP method, the recommended limits for microbiological monitoring in clean areas during operation involve the use of the settle plate technique for 4 hours at Grade D level (areas where

pharmaceutical products are not directly exposed to air). The established warning limit is 84 cfu/4h., and the action limit is 107 cfu/4h., which closely aligns with the 100 cfu/4h criteria. Therefore, it is advisable to establish criteria that do not exceed 100 cfu per 4 hours, in accordance with pharmaceutical regulations.

Derived from the Index of Microbial Air Contamination (IMA) [12], the one-hour settle plate method revealed counts ranging from 1 to 59 cfu/h. Applying the IMA criteria, these counts do not exceed 50 cfu per hour, indicating a fair class. The established warning limit is 45 cfu/h, and the action limit is 59 cfu/h, closely aligned with the 50 cfu/h criteria. Therefore, it is recommended to establish criteria that do not exceed 50 cfu/h, in accordance with the IMA.

However, the active air sampler method in these skincare facilities cannot comply with the guidelines from EU GMP [8] which recommends limits at Grade D level of 200 cfu/m^3. This is evident as the airborne microbial assessments conducted in each skincare factory revealed total microbial counts ranging from 85 to 252 cfu/m^3. The established warning limit is 282 cfu/m^3, and the action limit is 341 cfu/m^3, exceeding the value specified by the EU GMP method for Grade D level. Despite the fact that air sampling devices offer the advantage of providing more detailed information on microbial quantities compared to the basic method of placing agar plates, active air sampling is not commonly employed in most cosmetics factories due to its high cost (150,000 - 300,000 Baht). Therefore, the study recommends a suitable method for microbial sample collection in cosmetics factories, suggesting the use of the settle plate method for both 4 hours and 1 hour.

The statistical analysis and risk assessment underscore the importance of selecting cosmetic product groups for airborne microbial control. This stems from the notably high total microbial count observed in the production area for hair care products. However, the risk assessment maintains an acceptable level due to the low risk of microbial contamination in certain products, such as hair dye, containing substantial amounts of ammonia and hydrogen peroxide. Consequently, the recommendation is to prioritize cosmetic product groups that necessitate microbial control in the air, with a specific focus on collecting samples from high-risk products, in accordance with ISO 29621 [6] standards, particularly within the domain of skincare cosmetics.

Conclusions

The assessment of airborne microbes in cosmetics manufacturing factories 1, 2, 4, and 5 revealed variations in microbial counts, highlighting differences in contamination levels across these facilities. Despite these levels, the overall risk of contamination is manageable according to the risk matrix. Factory 3, producing low-risk hair dye products with high hydrogen peroxide levels, does not require stringent microbial control measures.

Routine operations can rely solely on testing for total microbial counts, as neither fungal nor Gram-negative bacterial counts were observed. Predominant bacteria, identified as Gram-positive cocci ranging from 62% to 78%, were consistent with those commonly found on human skin. If source verification is necessary, selective media for Gram-positive bacteria can be employed, as indicated in the study.

Given the quasi-field experimental nature of this study within active production facilities adhering to GMP for cosmetics, the influence of environmental factors (temperature, humidity, and wind speed) on microbial air quality control, as per GMP guidelines, might not be prominently discernible.

The establishment of control charts in alignment with statistical principles resulted in action limits set at 59 cfu/h, 107 cfu/4h, and 341 cfu/m^3 for the respective methods. Adherence to monitoring criteria in cosmetics manufacturing facilities should follow specified limits for airborne microbial counts, ensuring they do not surpass 100 cfu/4h (Grade D for sterile medicinal products) or 50 cfu/h (fair IMA level). Active air sampling is infrequently utilized in the majority of cosmetics factories.

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