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Environmental Microbial Monitoring and Risk Assessment of Cleanrooms - A Case Study in Medical Device Pilot Plant

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Abstract

The objective of this study is to determine the risk of environmental microbial contamination in the cleanrooms of a pilot plant that produced sterile microneedles-a type of medical device. Typically, microbial contamination comes from the human body, equipment, raw materials, and production environment. Environmental microbial monitoring in the production areas is frequently overlooked for a variety of reasons, including cumbersome and time-consuming procedures, inadequate facilities, and so on. Therefore, we intend to focus on this subject in the study. The environmental microbial monitoring program was established and implemented in the production area in nine steps: 1) determine risk areas, 2) select samples, 3) determine frequency and conditions, 4) assign persons, 5) select methods, 6) determine control limits, 7) analyze results and discussion, 8) investigate out-of-limit results, and 9) propose corrective actions and risk assessment. In addition, the bioburden of microneedle products was also part of this program. Data on airborne microorganisms were collected by the environmental microbial monitoring program from various risk points inside the pilot plant. This program's results were all data for determining risk assessment parameters. We generated a risk management system for this plant by using the guidelines of ISO 14971 and selecting three parameters to evaluate the risk: the likelihood-contamination rate, impact-bioburden, and deviation factors-out-of-control results of various parameters (consisting of temperature, humidity, differential pressure, and swab test.) in cleanrooms are the deviation. Besides, the process chart for risk control for environmental microbial contamination was established and used to facilitate the risk control of operators. This study aims to provide guidelines for microbial environmental monitoring and risk assessment in medical device pilot plants in accordance with ISO 13485 requirements.

Keywords : bioburden; environmental monitoring; ISO 13485; medical device; risk management

Introduction

A microneedle is a sterilized medical device (MD). The product must be free of microbiological contamination, safe to use, and stable, just like other sterile MDs such as a syringe, blood lancet, and so on. The production proceeds in a cleanroom where microbiological contamination by relevant factors is possible. The main factors causing contamination during operation, in descending order of risk, are raw materials (including water), equipment, personnel, and airborne environment [1, 2].

The fundamental principles of Good Manufacturing Practice (GMP) [3] used to prevent microbial contamination are cleaning, disinfection, and monitoring. Generally, in a factory, cleaning and disinfection are often well planned, while environmental monitoring (EM) does not have a clear action plan. However, a work environment control plan is necessary to implement the quality management system for medical devices

mandated by ISO 13485 [4] and prevent product contamination. Most importantly, the Food and Drug Administration (FDA) mandates that MD companies in several nations, including MD pilot facilities in Thailand, get GMP or ISO 13485 accreditation.

From the foregoing, we aim to examine the direct impact of airborne microorganisms on products and other factors likely to come into direct contact with products, such as equipment, personnel, and other contact surfaces. Therefore, the microbial EM program is established and applied to the pilot plant. Furthermore, the parameters, such as the number of samples, frequency, method, control limit, etc., that impact the program shall be specified. Any problems encountered during operation shall be corrected and prevented from recurring. The analysis data is required to establish criteria for contamination risk assessment. Finally, the risk assessment system can be fully implemented in this plant.

This paper aims to provide guidelines to support non-microbiologist researchers for microbial EM and risk assessment in MD pilot plants or starting production plants in accordance with ISO 13485 requirements.

Methodology

1. Microbiological environmental monitoring

Microbiological EM is a means of demonstrating acceptable microbiological quality in a controlled environment and detecting changes in time. It involves the collection of data relating to microbial numbers recovered from samples of air, surfaces, and people in the cleanrooms [2]. In this study, an EM program was developed and implemented in the plant to detect trends in microbial population changes and microbial growth within cleanrooms. The airborne samples were collected under production with a full staff to obtain the correct data and to collect all data in order to determine the risk control measures for microbial contamination in the products. In addition, this program also includes product sampling for the bioburden test. The application of the EM program came from JPAC [5]. It consists of 9 steps as follows:

Step 1 Determination of risk areas

All production areas in the pilot plant are in cleanrooms with two quality levels, class 10000 for production areas (such as storage of raw materials, molding, and assembling) and class 100000 for packaging areas (such as storage and preparation of packaging materials, packing). Both classes' names came from FED-STD-209E [6] which replaced by ISO 14644-1 [7], in which classes 10000 and 100000 are equivalent to ISO 7 and 8 of ISO 14644-1. Although a cleanroom helps to control the particles and microorganism counts not exceeding the controlled limits, there is still a risk that the number of microorganisms in the room may be out of control. The causes can be attributed to a variety of factors, including poor cleanliness, unauthorized entry, excess storage, introducing foreign objects into the area, abnormalities in the control system, and more. For this reason, the cleanroom also needs to assess the risk of contamination.

Step 2 Selection of samples and methods

According to EU GMP guidelines [3], microbial EM can be performed using four detection methods: air sample, settle plate, contact plate, and glove printing. The first two methods are the main methods for airborne microbial detection in the EM program. We chose the surface swab method instead of the last two methods. The steps of the swab test are according to ISO 18593 [8].

Volumetric air sampling is an active method operated by an air sampler to find the number of microorganisms per cubic meter of air. This method is applicable when the microbial concentration is not very high.

The settle plate is a passive method by EU GMP [3] that uses a petri dish (9 cm) with an agar medium opened and exposed to air for 4 hours. This method is valid and widely used. However, the method from the Index of Microbial Air Contamination (IMA) by C. Pasquarella, O. Pitzurra, and A. Savino [9] describes a method to determine airborne microbial contamination in at-risk environments by measuring the IMA. It stated that a Petri dish 9 cm in diameter containing plate count agar (PCA) is left open to air according to the 1/1/1 scheme (1 h, 1 m from the floor, at least 1 m

away from walls or any relevant physical obstacle). The microbial counted as CFU after 48 hours of incubation at 35±1°C. The number of CFU is the IMA. The IMA method is similar to the 4-hour settle plate method but saves monitoring time.

A swab test is a method for detecting microorganisms on surfaces in CFU per 100 cm^2, such as equipment, tables, doors, walls, hands or gloves, gown coats, etc. It uses a sterile stick with cotton or synthetic material at the tip. Sampling was performed by vigorously sweeping the surface to collect samples. Afterward, the swab was stored in a sealed sterile container and sent to a microbiology laboratory.

The bioburden test is the procedure for identifying microorganisms living on the surface of a product before sterilization. This result could explain the likelihood of microbial contamination in the current environment. The number of microbial species found in this test will reflect the effectiveness of the EM program at that time. The causes of contamination can be unsanitary preparation, packing, or storage, or the non-hygienic practices of staff. In addition, the bioburden results would determine the optimal gamma radiation dose for product sterilization. Excessive gamma radiation can affect product and packaging quality. On the other hand, if the gamma radiation dose is too low, it can lead to the incomplete elimination of microorganisms [10].

Step 3 Determination of frequency and monitoring conditions

We should consider the possibility of microorganisms accumulating within a cleanroom before determining the optimal frequency for the EM program. For example, the frequent production, the possibility of contamination in the manufacturing process, the history of contamination, etc. [2]. We operate the EM program in two stages. At the initial stage, there are two times for environmental microorganism monitoring and one time for the bioburden test. In the routine operation, every production from February 2021 to May 2022 took place a total of six times (because of the infrequency of microneedle production in the pilot plant). The monitoring frequency may be tightened or reduced depending on past results. However, the frequency will be reviewed appropriately by considering the historical data, including adjusting the conditions for monitoring again.

If possible, the monitoring should take place during production at different times when raw materials and all facilities have remained in the production area with all working staff. We should identify the risk points in each cleanroom area and monitor each risk point [11]. In addition, other monitoring activities related to cleanroom functionality should be done too, for example, airborne particle counts, HEPA filter integrity testing, air change rate calculations, air pressure difference, temperature and humidity, etc. [12].

Step 4 Assignment of responsible persons

The person in charge of the EM program should have relevant knowledge in the three areas: microbiology, environmental management, and quality management systems. It is difficult to recruit qualified personnel in all areas. However, the most required knowledge for this work is microbiology. Therefore, the assignment of a microbiologist to manage this program is ideal. Additional training can be another source of information. In addition, the appointed person should have other necessary qualifications, such as experience in this job.

Step 5 Selection of microbiological analysis methods

All microbial testing used in the EM program is the standard method. For the EM, there are two types of microorganisms analyzed: the aerobic plate count (APC) and the fungal count (yeast and mould). Both microorganisms perform in the on-site microbiology laboratory. Tryptic Soy Agar (TSA) is a medium culture for APC culture, and use DG-18 is the medium culture for fungi. All cultural media preparation was carried out in accordance with ISO 11133:2014 [13].

All product samples were sent to an external microbiological laboratory to test for bioburden. The test method is according to ISO 11737-1:2018 [14] to analyze microorganisms on the surface of the non-sterile microneedle samples.

Step 6 Determination of the control limit

The EU GMP Guidelines for Therapy Medicinal Products [3] have recommended maximum limits that are used to control environmental microorganisms in clean areas. It classified clean areas leveled by microbial cleanliness into four grades: A, B, C, and D, as shown in Table 1, where "A" denotes operations in the at-risk area, "B" for operations in the sterile area, "C" for the control area, and "D" for the support area. Table 2 is the comparison table of cleanroom classifications based on air changes per hour between ISO 14644-1:2015 [7], FED-STD-209E [6], and EU GMP [3]. This table states that grades C and D cleanrooms are equivalent to classes 10000 and 100000 of this plant, respectively.

Table 1 Microbiological cleanliness levels in operation [3]

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	-

Table 2 Comparison of cleanroom classifications [3, 6, 7]

Cleanroom Std.	Cleanroom Classification Guidelines					
ISO 14644-1	Class 3	Class 4	Class 5	Class 6	Class 7	Class 8
FED-STD-209E	1	10	100	1000	10000	100000
EU GMP	-	-	A / B	-	C	D

In the early adoption of the EM program, the control limits of the air sampler and settle plate from Table 1 can be used as the tentative criteria for evaluating airborne microbial counts in both cleanrooms. In the case of swab tests, we use them to assess the efficiency of cleaning and sanitation by using the control criteria of microorganisms found to be less than or equal to 100 CFU/100 cm^2 (adapted from Griffiths 2016) [15]. (See Table 3).

Table 3 The tentative control criteria used in the EM Program

Class of cleanroom	Air Sample (cfu/m^3)	Dia. 90 mm. Settle Plate (cfu/4h)	Swab test on surface (cfu/100cm^2)	Swab test Glove & cloth (cfu/100cm^2)
10000 (C)	100	50	100	100
100000 (D)	200	100	100	100

To establish a risk management plan for airborne microbial contamination in this plant, it is necessary to determine the contamination rate (CR), where CR is a key parameter used to assess the likelihood of microbial contamination. We can calculate CR as a percent by referring to the formula in Sandle's Numerical Approaches to Risk Assessment, T (2019) [2], as follow:

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

The settle plate count means microbial count, the area of a product is 12.57 sq. cm, the Petri-dish area is 63.64 sq. cm, the time of product exposure is 2.5 min, and the time of settle plate exposure is four hours (EU GMP) or one hour (IMA). Additionally, the IMA method [9] in Table 4 divides the IMA into five classes. The table specifies microbial content as IMA, CFU/dm^2/h, quality class, and risk status. The values in this table can be used to compute the % CR of airborne microorganisms.

Table 4 The index of microbial air contamination (IMA) [9]

IMA value	cfu/dm^2/h	Quality class	Risk status
0-5	0-9	Very good	Very low
6-25	10-39	Good	Low
26-50	40-84	Fair	Medium
51-75	85-124	Poor	High
$\geq$ 76	$\geq$ 125	Very poor	Very high

Step 7 Data for trend analysis and discussion

At the start of the EM program, we monitored environmental microorganisms in the plant twice, in November 2020 and February 2021. We used a settling plate and air sampler for airborne microbial monitoring and the swab test on many surfaces that may be contamination risk points. In routine operations, we used the IMA method for airborne microbial monitoring. Four monitoring areas in the plant were defined: the class 10000 cleanroom, the class 100000 cleanroom, the changing rooms, and the corridor. Airborne microorganisms are monitored every time in pre- and post-production to compare the differences. All activities have been done six times from February 2021 until May 2022. We can evaluate the results from the starting stage by comparing the data with the tentative control criteria in Table 3 and the data results from the

routine operation by comparing them with the control criteria in Table 4.

Step 8 Investigation of out-of-limit results

From step 7, if some data were out of the control limit, an investigation of the root cause should act quickly. We should consider all defects that could be the causes and provide appropriate countermeasures immediately.

Step 9 Corrective actions and risk assessment

All defects detected in EM programs should be effectively corrected every time. By establishing and implementing a risk assessment system in the plant, we can reduce environmental microbial contamination as much as possible in the long run. There are several ways to approach risk assessment. We choose to use the guidelines of ISO 14971:2019 - Application of Risk Management to Medical Devices [16], which will be explained in the next topic.

2. Establishment of a risk assessment system

Referring to the regulations of the U. S. FDA [17], microneedle products are MDs for use in specified areas of the human body. It is an instrument with technological features, having many tiny needles, tips, or pins on the surface to create many small puncture holes in the skin. They used them in various biomedical areas such as drug delivery systems, disease wound repair, and cancer therapy. Because microneedles come into direct contact with the body, product safety must be recognized in order to avoid infection or dangers in use to the user. Therefore, it is necessary to control the various characteristics related to product safety, such as sterility, surface roughness, material properties, energy sources, etc.

In designing a system of environmental microbial contamination prevention for this plant, we developed it by following the guidelines of ISO 14971:2019 [16]. This standard specifies the five steps of the risk management process: 1) risk assessment, including risk analysis and risk evaluation; 2) risk control; 3) evaluation of overall residual risk; 4) risk management review; and 5) production and post-production activities.

Step 1 Risk assessment

The steps for risk assessment comprised a risk analysis and a risk evaluation, as follows:

Step 1.1 Risk analysis

Microbial contamination can cause a before-sterilized microneedle product to have an excessive amount of microorganisms that lead to an inadequate sterilization dose, making this product dangerous when used due to the risk of infection. To estimate the risk of microbial contamination, we shall assign the possibility of contamination, called "Likelihood," and the consequences of this contamination, called "Impact." The likelihood relates to the contamination rate from the airborne environment, calculated as a percentage of contamination rate (% CR), while the impact means the microbial load count found on the before-sterilized microneedle products.

Step 1.2 Risk evaluation

We need to create a risk matrix based on the likelihood-impact relationship to assess the risk of airborne microbial contamination. In this case, the likelihood is CR% and the impact is bioburden. We can use the maximum values in each IMA class from Table 4 to calculate % CR. The results of the % CR values can be used to generate the likelihood tables for the contamination at the five levels shown in Table 5 below.

Table 5 The five likelihood levels of microbial contamination

Likelihood Level	% Contamination rate (%CR)		Score Level
	From Max. of IMA	From Max. of CFU/dm^3/h	
Improbable	$\leq$ 4 %	$\leq$ 7 %	1
Remote	5 - 20 %	8 - 32 %	2
Occasional	21 - 41 %	33 - 69 %	3
Probable	42 - 62 %	70 -102 %	4
Frequent	$\geq$ 63 %	$\geq$ 103 %	5

Referring to ISO 11137-2 [18], the impact rating can be determined by considering the sterilization dose of gamma-ray on the product. If using 25 KGy, the microbial count from the bioburden test shall be less than or equal to 1000 CFU, and if using 15 KGy, it shall be less than or equal to 1.5 CFU. As a result, the number of microorganisms on the product shall be between 1.5 and 1,000 CFU. We can use this range of numbers to establish the impact table of the bioburden products by

dividing the impact by the five levels within this range (see Table 6).

Table 6 The five levels of impact from the bioburden product

Impact Level	Bioburden Result (CFU)	Score Level
Negligible	< 1.5*	1
Minor	1.5 - 10	2
Moderate	> 10 - 100	3
Major	> 100 - 1000*	4
Critical	> 1000	5

** Refer to ISO 11137-2, at Topic 9.2.1.1 and Topic 9.4.1.1*

We can multiply each likelihood and impact level to establish the risk matrix shown in Figure 1. 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.

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

Figure 1 The risk matrix by multiplying likelihood and impact

Step 2 Risk control

In the determination of risk control measures to cover all factors that affect the risk, in addition to the likelihood and impact mentioned in Step 1.2, the parameters related to the cleanliness measurement within the cleanroom are also factors that shall be taken into account. There are two essential parameters: the swab tests and the controlled conditions within the cleanroom, including temperature, relative humidity, and different pressures inside and outside the cleanroom. The risk of deviation from the control value of these two parameters is called the "Deviation Factor." We can calculate the deviation factor in percentage, as shown in the following calculating formula:

1) Calculation of deviation factor from swab test

$$\%\text{Deviation}_{\text{Swab}} = \frac{\text{Sum of swab test results deviating from the control value}}{\text{The total chosen risk point for the swab test}} \times 100$$

2) Calculation of the controlling parameter (CP) in cleanrooms

$$\%\ \text{Deviation}_{\text{CP}} = \text{Sum of \% deviating from the control value of T, RH, and DP}$$

By referring to ANSI/ASQ Z1.4 [19], we use the acceptance limit of the summation of percent deviation from the swab test plus CP at a maximum of 3% and use this figure to classify the level of deviation risk shown in Table 7.

We combine the likelihood, impact, and deviation factors to establish the risk control table by multiplying the scores of these factors at the same level. The total scores are between 1 and 125. The risk consists of five levels, and each score level is in the appropriate range (see Table 8). We will use this risk control table in the pilot plant to check the status of microbial contamination risk.

Table 7 The level of risk from deviation factor

Deviation Level	Percent acceptance of deviation	Score Level
Negligible	$\%\ \text{Deviation}_{\text{Swab}}$+$\%\ \text{Deviation}_{\text{CP}}$ < 1%	1
Minor	$\%\ \text{Deviation}_{\text{Swab}}$+$\%\ \text{Deviation}_{\text{CP}}$ 1-2%	2
Moderate	$\%\ \text{Deviation}_{\text{Swab}}$+$\%\ \text{Deviation}_{\text{CP}}$ >2-3%	3
Major	$\%\ \text{Deviation}_{\text{Swab}}$+$\%\ \text{Deviation}_{\text{CP}}$ >3-5%	4
Critical	$\%\ \text{Deviation}_{\text{Swab}}$+$\%\ \text{Deviation}_{\text{CP}}$ >5%	5

Table 8 Risk control table of environmental microbial contamination

Risk Rating	Possibility of Hazard Situation	Risk Score
Very low risk	No contamination happens to the products	1 - 4
Low risk	Difficult to contaminate the products.	>4 - 8
Moderate risk	There is still a chance of product contamination	>8 - 27
High risk	There is a risk of product contamination easily.	>27 - 64
Very high risk	Product contamination can occur at any time	>64 - 125

Step 3 Evaluation of Overall Residual Risk

We have established the risk control process for environmental microbial contamination in the plant (see Figure 2) for operators to use as the guideline to continuously monitor and control the overall residual risk in the plant. After that, performance results are collected and used to re-assess residual risks in pilot plants at least once a year.

Step 4 Risk management review

It needs to collect the performance records from the EM program and the relevant information to summarize the result and any problems or limitations between operations. After that, we should send all the information to management for review in three aspects: 1) the suitability of operation in the system, 2) the acceptability of overall residual risk, and 3) taking appropriate and timely corrective measures, including verifying data during and after production. The responsible person should take action in accordance with the opinions and recommendations received from the risk management review appropriately.

Step 5 Production & post-production activities

Besides the sources causing the risk of microbial contamination in the environment directly, we should not ignore the other sources that involve microbial contamination too, such as sources of raw materials or packaging in their supply chain and the chances of cross-contamination from storage, transportation, and use. An instruction manual for collecting and reviewing all information relevant to every activity during and post-production is needed. Moreover, it must have other necessary data, such as technical data and specifications of the microneedle, related reference standards, and others. Statistical techniques should be used to analyze data trends. The complicated work should be documented as a standard operating procedure (SOP). The EM program should be improved in light of the current hazard situation, and risk control measures should be added (if necessary).

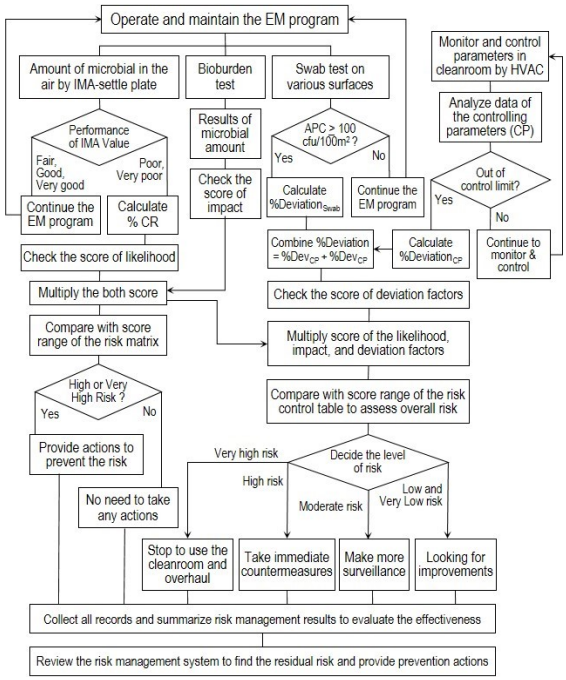

Figure 2 Process of risk control for environmental microbial contamination

Results and Discussion

1. Results from the EM program

At the initial stage of the EM program, the results from the method of the settle plate and the air sampler could be concluded, as shown in Tables 9 and 10, respectively. Table 9 showed the results of APC from the settle plate in both cleanrooms. In the first result, we monitored six risk points for class 10000 cleanroom and nine for class 100000. In the second, the monitoring was done at two and six risk points, respectively. Values of APC in minimum, maximum, and mean were shown in the table and pass evaluations. In addition, we found a few fungi in both cleanrooms in the first monitoring, so there is no need to monitor them a second time. The bacterial types in the air were identified and found to be gram-positive cocci at 82%, gram-positive bacilli at 5%, gram-negative bacteria at 6%, and mold at 7%. Many of the gram-positive cocci bacteria in the air of occupied areas (such as *Staphylococcus* spp.) came from the skin of personnel [20].

Table 9 Evaluation of APC in Cleanrooms by Settle Plate (4 h)

Class of cleanroom	Sampling date	APC by Settle plate (4h)				Results
		Control limit	Min. CFU	Max. CFU	Mean CFU	
10000	26/11/20	50	0	7	3.7	passed
	23/02/21		3	4	3.5	passed
100000	26/11/20	100	0	3	1.4	passed
	23/02/21		2	9	5.0	passed

Table 10 showed the results from the air sampler. In the first result, we monitored one risk point in the class 10000 cleanroom and two in the class 100000 cleanroom. In the second, they were two and one risk point, respectively. All results of APC passed the control criteria in Table 3. In the case of the fungal test, the results were small counts of mould detected in each cleanroom (between 0 and 7 CFU).

Table 10 Evaluation of APC in Cleanrooms by Air Sampler

Class of cleanroom	Sampling date	APC by Air Sample				Results
		Control limit	Min. CFU	Max. CFU	Mean CFU	
10000	26/11/20	100	18	18	18.0	passed
	23/02/21		28	61	44.5	passed
100000	26/11/20	200	7	60	33.5	passed
	23/02/21		34	34	34.0	passed

From the swab test results, the eighteen samples were from many surfaces within both cleanrooms, such as tables, door handles, storage cabinets, plastic bags, blister packs, cartons, gown coats, and equipment. All results found small amounts of APC between 0 and 6 CFU on each surface.

We also monitored airborne bacteria outside the cleanroom by the settle plate and found the max value of APC to 96 CFU and fungi to 116 CFU. In addition, on the sleeves of gown coats hanging in the closet of the changing room, we found the max value of APC to be 129 CFU. It seems that gown coat was a high-risk factor for contamination.

We compared the airborne microbial monitoring method between the 4-hour settle plate and the 1-hour settle plate (IMA) to check for differences in results. The number of ten samples per method was picked up from the same point in the cleanroom, changing room, and outside the cleanroom to find APC in the air. We used ANOVA Single Factor to analyze the APC data from both methods. This statistical analysis result stated that the APC values were not significantly different. So, we could use the IMA method instead of the 4-hour settle plate in routine operations.

We were unable to identify the type of bacteria from the bioburden test results because none of the thirty microneedle samples had any microorganisms visible.

In routine operations, the airborne microbial monitoring by IMA had been done six times from February 2021 until May 2022. The trends in the six monitoring results were shown in Figures 3 and 4 as follows: We could conclude from Figure 3 that most of the microbial counts obtained by monitoring in both cleanrooms met the criteria of "good" to "very good," and some results met the criteria of "fair" (IMA criteria [9] in Table 4). Based on the microbial monitoring trend in both cleanrooms, most microorganisms in post-production were lower than in pre-production, except in the first monitoring, which gave the opposite result. Perhaps it is because raw materials and packaging materials were stored a lot during that time. Figure 4 showed the trend of airborne microorganisms outside the cleanroom. All results met the "good to fair" criteria.

In Figure 5, the red dotted line graph was the average APC value of the four areas obtained from the swab test. This graph showed the APC increase trend of the swab test and found that the APC in a class 10000 cleanroom was higher than in the other areas. The reason may be due to incomplete cleaning and disinfection of these surfaces. This trend may further increase the risk of microbial contamination.

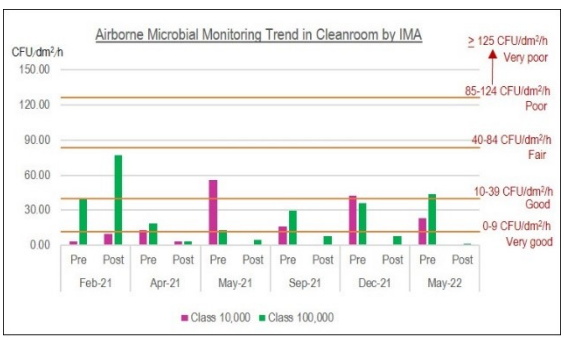

Figure 3 Airborne Microbial Monitoring Result in Cleanroom

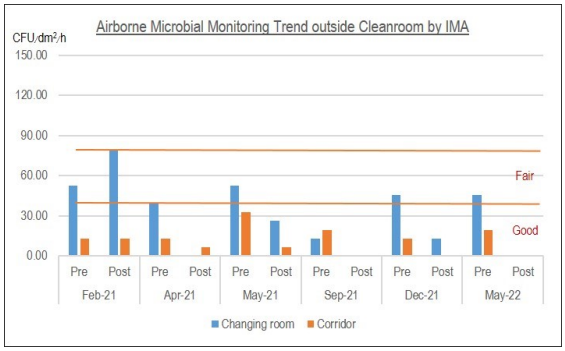

Figure 4 Airborne Microbial Monitoring Result outside Cleanroom

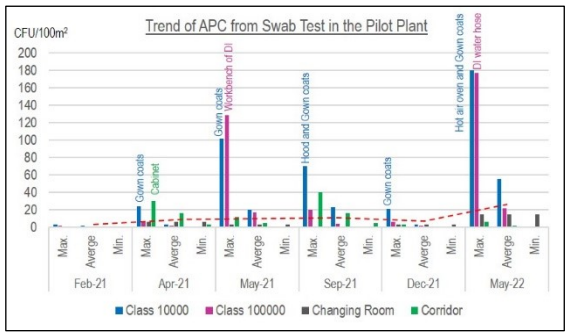

Figure 5 Results of Swab Test in Four Areas of the Pilot Plant

From the operation of the EM program, the results of all airborne microbial tests in the cleanrooms were within the control limit, except some swab test results were out of control values, especially on the surfaces of the table and pipe of the DI, the hem of gown coats, and some device surfaces, which were trending upward. These were already correct or need to be changed, such as the creation of SOPs for operators to serve as cleaning guidelines in each area.

Microbiological quality control represented a cornerstone in the MDs production process. With evolving regulatory requirements, products of greater complexity were elevating the challenges related to maintaining microbiological integrity. The fundamental role of GMP needs to be closely monitored, especially the EM program. EM can be achieved in practice with the long-term trend for validation studies and risk assessment [2, 21, 22].

2. Results from risk assessment

2.1) The likelihood score

In 2021, airborne microorganisms were monitored five times each in class 10000 and class 100000 cleanrooms using the IMA method. All microbial results were calculated as % CR by Sandle's formula, and the % CR values appeared in Table 11. The max value of % CR in the table was used to check the likelihood score with Table 5 and it was found that the scores for the cleanroom classes 10000 and 100000 were 2 and 3, respectively.

Table 11 The Likelihood Score in the year 2021

Class of Cleanroom	% CR from Sandle's Calculation Formula					Likelihood Score
	Feb-21	Apr-21	May-21	Sep-21	Dec-21	
10000	5.18	6.90	29.34	8.63	22.44	2
100000	40.73	9.78	6.90	15.01	18.98	3

2.2) The impact score

There were no microorganisms found in the thirty samples of the bioburden test. We can find the impact score in Table 6, and the impact score of both cleanrooms was 1.

2.3) The deviation score

2.3.1) Deviation from the swab test

4 of 46 swab test results this year in cleanroom class 10000 were out of control, resulting in an 8.7% deviation. In the cleanroom class 100000, 2 of 73 tests were out of control, resulting in a 2.74% deviation.

2.3.2) Deviation from temperature, relative humidity, and different pressure

The control criteria for temperature in a clean room were 22.2±2.8°C, the relative humidity is between 30-65% [23], and the pressure difference of the class 10000 cleanroom is more than 12.5. Pa. In cleanroom class 100000, we used the criteria of 5-20 Pa according to ISO 14644-4. Figures 6 and 7 showed the values of temperature (T), relative humidity (RH), and air pressure difference (DP), including the trend of their control results, in which all the data are under control criteria. Both figures showed the monthly average values of temperature, relative humidity, and pressure differences in both cleanrooms (a total of 12 times). It turns out that each parameter was under control every month.

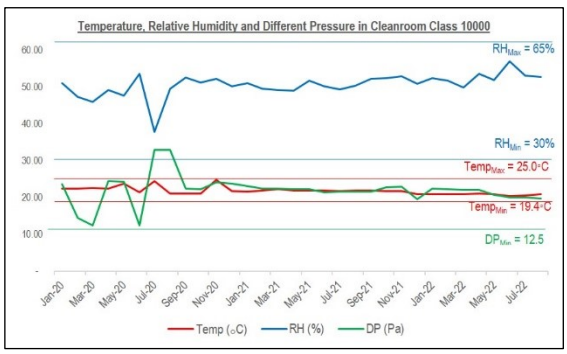

Figure 6 Trend of T, RH, and DP in Cleanroom Class 10000 at Operation Condition

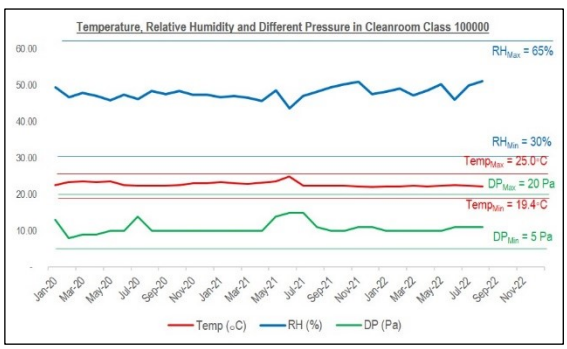

Figure 7 Trend of T, RH, and DP in Cleanroom Class 100000 at Operation Condition

Table 12 showed the sum of the % deviations in class 10000 and 100000 cleanrooms were 8.70% and 2.74%, respectively. It can be found that the deviation score in Table 7, and the scores of each cleanroom were 5 and 3, respectively.

Table 12 The Deviation Score in the year 2021

Control Factors in Cleanroom	No. of times to monitor		No. of times out of control		% Deviation	
	C.10000	C.100000	C.10000	C.100000	C.10000	C.100000
Swab test	46	73	4	2	8.70%	2.74%
Temp (T)	12	12	0	0	0.00%	0.00%
Humidity (RH)	12	12	0	0	0.00%	0.00%
Diff Pres (DP)	12	12	0	0	0.00%	0.00%
Sum of % deviation					8.70%	2.74%
Deviation score					5	3

2.4) The risk score

The result of multiplying the likelihood score, the impact score, and the deviation score was called the risk score. From Table 13, the risk scores of class 10000 and 100000 cleanrooms were 10 and 9, respectively.

Table 13 The Risk Score in the year 2021

Cleanroom	Likelihood Score	Impact Score	Deviation Score	Risk Score	Risk Rating
Class 10000	2	1	5	10	Moderate
Class 100000	3	1	3	9	Moderate

There were no case studies of microbial risk assessment in MD; there were only cases in pharmaceuticals [24-26].

Conclusions

The microbial EM program was established and implemented in the production area with nine steps (applied from JPAC [5]):

1. Determination of risk areas
2. Selection of samples and methods
3. Determination of frequency and monitoring conditions
4. Assignment of responsible persons
5. Selection of microbiological analysis methods
6. Determination of the control limit
7. Data for trend analysis and discussion
8. Investigation of out-of-limit results
9. Corrective actions and risk assessment

Figure 1 Flow chart of the EM Program

We generated a risk management system for this plant by using the guidelines in ISO 14971:2019 and selecting three parameters to evaluate the risk: the likelihood, impact, and deviation factors. In this study, the percentage of contamination rate is the likelihood, the number of microorganisms from the bioburden test is the impact, and the out-of-control results of various parameters (consisting of temperature, humidity, differential pressure, and a swab test) in cleanrooms are the deviation.

The risk evaluation result of the plant in 2021 was a moderate risk that was acceptable. A critical point of the risk came from the deviation factor of the swab test, especially from the gown coats. This risk factor was

already corrected and did not find a recurrence. The EM program continued to be followed today, including accurate and complete records of operating results to maintain an effective risk management system at all times. All performance results would be analyzed and concluded for review by management in three aspects: 1) suitability of correction measures, 2) overall residual risk remaining in the plant, and 3) more activities to do. Many outputs from the review should be applied to improve or develop the system.

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